USE OF EPOTHILONES IN THE TREATMENT OF NEURONAL CONNECTIVITY DEFECTS SUCH AS SCHIZOPHRENIA AND AUTISM

The present invention relates to the use of epothilones for manufacturing a medicament for use in the treatment of psychotic disorders thought to be associated with neuronal connectivity defects, in the absence of obvious anatomical, degenerative, or proliferative anomalies.

Psychotic disorders are disorders that are predominantly characterized by an impairment of mental functioning to the extent that it interferes grossly with an individual's ability to meet the ordinary demands of life.

Psychotic disorders currently thought to result from disorders in neuronal connectivity include, and are not limited to, schizophrenia, schizophreniform disorder, schizoaffective or delusional disorder, and autism (Andreassen NC, Brain Res. Rev. 2000; 31:106-12; Francke *et al.*, Neuron, 2003, 39, 205-216; Jamain *et al,* Nature Genetics, 2003, 34, 27-28.l).

Schizophrenia is any of a group of psychotic disorders usually characterized by withdrawal from reality, illogical patterns of thinking, delusions and hallucinations and accompanied in varying degrees by other emotional, behavioral or intellectual disturbances. A lifelong chronic mental illness, schizophrenia exhibits positive and negative symptoms, with an onset in young adulthood and deterioration from the previous level of functioning. Positive symptoms reflect a distortion or excess of normal functions (eg, disorganized speech, delusions, and hallucinations). Negative symptoms, on the other hand, reflect a restricted range of normal behavior and emotions (eg, apathy, paucity of speech and incongruity or flattening of emotional responses). Schizophrenia can be presented in various forms depending on the symptoms and signs. The varieties of schizophrenia include paranoid schizophrenia, hebephrenic schizophrenia, catatonic schizophrenia, and undifferentiated schizophrenia as well as post-schizophrenic depression, residual schizophrenia, simple schizophrenia and unspecified schizophrenia.

Neuroleptic agents or drugs (i.e. "neuroleptics"), also called anti-psychotics or major tranquilizers remain the treatment of choice for schizophrenia, certain organic

central nervous system disorders, mental illnesses, and associated psychotic processes today. Currently, it is estimated that over 95% of schizophrenics are chronically maintained on neuroleptics. All known neuroleptics are dopaminergic blockers, having pharmacological actions similar to the action of chlorpromazine, an aliphatic phenothiazine derivative also known as Thiorazine.

Although the known anti-psychotic drugs have clear efficacy in the treatment of mental illness, they do not cure all symptoms and have a variety of side effects.

The invention derives from the discovery that epothilones can alleviate schizophrenia-related behavioural disorders in an animal model termed STOP deficient mice (Andrieux *et al.*: Genes Dev. 2002 Sep 15; 16(18): 2350-64).

The natural products Epothilones A and B as well as some of their synthetic derivatives have recently found interest in connection with the treatment of cancer. Thus, WO 98/22461, WO 99/07692, DE 198 21954, WO 99/02514, WO 99/67252, WO 00/50423, WO 02/21712, WO 00/66589, WO 01/081341, WO 00/49021 and US 2003/0 203 929 deal with the synthesis of epothilone derivatives and for some of them with their use in the treatment of cancers. WO 03/074053 relates more specifically to the use of some epothilone derivatives to treat diseases involving degenerative or hyperproliferative processes, such as brain tumor, Alzeihmer's disease, multiple sclerosis and primary or secondary brain tumors.

A beneficial effect of epothilones has now been demonstrated in STOP deficient mice which display neuronal connectivity defects not associated with any detectable anatomical, degenerative or proliferative anomalies.

It has been observed, notably, that administration of epothilone D to STOP KO mice resulted in a decrease in the total number of shifts between activities indicating an alleviation of the activity fragmentation, characterizing untreated STOP deficient mice.

Furthermore, the maternal behaviour of STOP deficient female mice treated with epothilone D was re-established in the extent to which it was compatible with pup survival.

It has been observed that after D epothilone treatment, the synaptic vesicle density was increased in STOP KO mice by about 20% such an effect being similar to that obtained after a long-term neuroleptic treatment.

Accordingly, the invention provides alternative therapies for treating some Central Nervous System (CNS) disorders and mental illness, associated with neuronal connectivity defect, particularly schizophrenia and autism, said defects being not associated with any detectable anatomical, degenerative or proliferative anomalies.

According to a first aspect, the present invention is directed to the use of at least one epothilone or derivative thereof as an active ingredient, in particular in a therapeutically effective amount, for manufacturing a medicament for use in the treatment of disease(s) involving a neuronal connectivity defect.

In particular, the present invention is directed to the use of at least one epothilone or derivative thereof as an active ingredient, in particular in a therapeutically effective amount, for manufacturing a medicament for use in the treatment of schizophrenia and/or autism.

According to a second aspect, the present invention is directed to a method of treatment of disease(s) involving a neuronal connectivity defect comprising administering to an individual in need thereof a therapeutically effective amount of at least one epothilone or derivative thereof, in particular as defined according to the invention.

An embodiment of the method of the present invention comprises administering to an individual a therapeutically effective amount of at least one epothilone or derivative thereof, in particular as defined according to the instant invention, in a pharmaceutical composition comprising at least a pharmaceutically acceptable carrier.

Another embodiment of the method of the present invention comprises administering to an individual a therapeutically effective amount of at least one epothilone or derivative thereof, in particular according to the invention or a pharmaceutical composition thereof in combination with one or more agents useful in preventing or treating psychotic or psychiatric disorders, in particular such as neuroleptics.

As used herein, the term "disease associated with neuronal connectivity defect" refers to mental diseases currently thought to involve neuronal connectivity disorder, in the absence of obvious anatomical, proliferative or degenerative anomaly. Examples of such disorders include particularly schizophrenia and autism. In particular, the diseases considered according to the invention are different from progressive dementing disorders like Alzheimer, which involve neuronal degeneration.

As used herein, the term "schizophrenia" refers to a psychiatric disorder that includes at least two of the following: delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behaviour, or negative symptoms. Patients can be diagnosed as schizophrenic using the DSM-IV criteria (APA, 1994, *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition), Washington, D.C.).

"Negative" symptoms of schizophrenia include affect blunting, anergia, alogia and social withdrawal, which can be measured using SANS (the Scales for the Assessment of Negative Symptoms; see Andreasen, 1983, *Scales for the Assessment of Negative Symptoms* (SANS), Iowa City, Iowa).

"Positive" symptoms of schizophrenia include delusion and hallucination, which can be measured using PANSS (the Positive and Negative Syndrome Scale; see Kay *et al.*, 1987, Schizophrenia Bulletin 13:261-276).

"Cognitive" symptoms of schizophrenia include impairment in obtaining, organizing, and using intellectual knowledge which can be measured by the Positive and Negative Syndrome Scale-cognitive subscale (PANSS-cognitive subscale) (Lindenmayer *et al.*, 1994, J. Nerv. Ment. Dis. 182:631-638) or with cognitive tasks such as the Wisconsin Card Sorting Test.

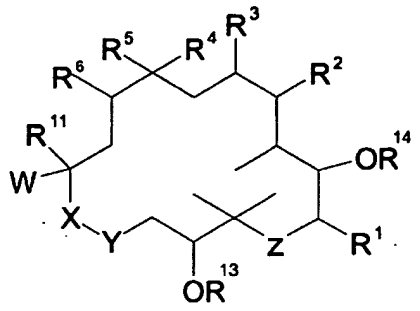
As used herein, the term "autism" refers to a state of mental introversion characterized by morbid self-absorption, social failure, language delay, and stereotyped behavior. Patients can be diagnosed as suffering from autism by using the DSM-IV criteria.

"Treating" or "treatment" as used herein refers to the treatment of a disease in an individual, which disease is associated with neuronal connectivity defect and includes:

(i) inhibiting the disease, i.e., arresting its development; or

(ii) relieving the disease, i.e., alleviating symptoms caused by the disease.

According to one embodiment, the used epothilone may be derivatives of epothilones of following formula (I):



(I)

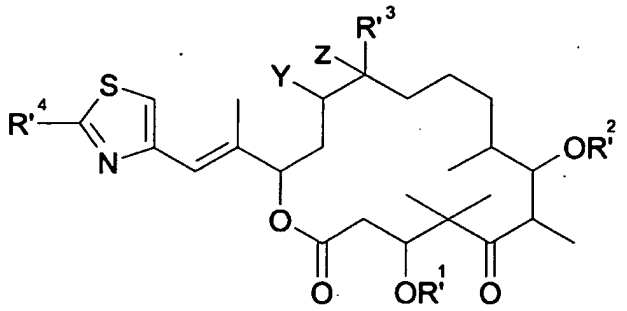
wherein:

- R^1 represents H, alkyl, alkenyl or alkynyl in C_1-C_6, aryl in C_6-C_{10}, aralkyl in C_7-C_{15},
- R^2, R^3 represents each H or form together C=C double bond,
- R^4 represents H, C_1-C_6-alkyl in particular CH_3, fluoro substituted C_1-C_6 alkyl in particular CF_3 or CFH_2,
- R^5 and R^6 form a C=C double bond or a three membered ring including O, S, NR^7, CR^8R^9 with R^7 being $C(O)R^{10}$, SO_2R^{10} and R^8, R^9, R^{10} being independently H, halogen, C_1-C_6 alkyl, C_6-C_{10} aryl, C_7-C_{15} alkaryl,
- R^{11} being H, C_1-C_6 alkyl, C_6-C_{10} aryl, C_7-C_{15} alkaryl, and in particular H,
- W represents $C(R^{12})$=CH, $C(R^{12})$=$C(CH_3)$, $C(R^{12})$=CF or a bicyclic aromatic/heteroaromatic radical preferably a 2-methylbenzothiazol-5-yl radical, or a 2-methylbenzoxazol-5-yl radical or a quinolin-7-yl radical, with R^{12} representing a heteroaromatic radical, preferably a 2-pyridinyl, a 2-substituted thiazol-4-yl or a 2-substituted oxazol-4-yl radical with substitution in 2-position by C_1-C_6-alkyl, pseudohalogen like CN or N_3, S-C_1-C_4-alkyl, O-C_1-C_6-alkyl, or C_1-C_6-alkyl substituted by OH, amino, halogen, pseudohalogen such as –NCO, -NCS, $–N_3$, O-(C_1-C_6)-acyl, O-(C_1-C_6)-alkyl or O-benzoyl,
- X-Y represents O-C(=O), O-CH_2 , CH_2-O, CH_2-C(=O),
- Z represents C=O, S, S=O, SO_2,

- R^{13} and R^{14} represents independently from each other H, C_1-C_6-alkyl, $(CO)R^{15}$ or C_{1-4}-trialkylsilyl, with R^{15} being H, C_1-C_6-alkyl, fluoro substituted C_1-C_6-alkyl, and

pharmaceutically acceptable salts thereof.

According to one embodiment, the used epothilone may be derivatives of epothilones of following formula (II):



(II)

wherein:

- R'^4 represents an C_1-C_6-alkyl or substituted C_1-C_6-alkyl with substituents as F, Cl, Br or I, pseudohalogen such as –NCO, -NCS, $–N_3$, NH_2, OH, O-(C_1-C_6)-acyl, O-(C_1-C_6)-alkyl or O-benzoyl,
- R'^1 and R'^2 are independently from each other H, C_1-C_6-alkyl, $(CO)R'^5$ with R'^5 being H, C_1-C_6-alkyl, C_1-C_6-fluoroalkyl or C_{1-4}-trialkylsilyl,
- R'^3 represents H, C_1-C_6-alkyl, halogen substituted C_1-C_6-alkyl, and
- Y and Z form either a C=C double bond or are the O atom of an epoxide

and pharmaceutically acceptable salts thereof.

Depending on the nature of the various subsistuents, the compounds of formula (I) and (II) may have several asymmetric carbon atoms. Unless otherwise mentioned or indicated, the chemical designation of compounds denotes the mixture of all possible stereochemically isomeric forms, said mixtures containing all diastereomers and enantiomers of the basic molecular structure.

The term addition salt as used hereinabove also comprises the solvates which the compounds of formula (I) and (II) are able to form and said solvates are meant to be

included within the scope of the present invention. Examples of such solvates are e.g., the hydrates, alcoholates and the like.

As used therein,

the term "alkyl" includes straight chained and branched hydrocarbon groups containing the indicated number of carbon atoms, typically methyl, ethyl, and straight-chain and branched propyl and butyl groups. Unless otherwise indicated, the hydrocarbon group can obtain up to 16 carbon atoms. The term "alkyl" includes "bridged alkyl". Alkyl groups can be substituted, for example, with hydroxy (OH), halogen, aryl, heteroaryl, cycloalkyl, heterocycloalkyl, cycloalkenyl, heterocycloalkenyl, amino ($N(R^b)_2$), and sulfonyl (SO_2R^b), wherein R^b is selected from the group consisting of hydro, C_1-C_6alkyl, cycloalkyl, aryl, and SO_2C_1-C_6-alkyl, or two R^b groups are taken together to form a 5- or 6-membered ring.

The terms "cycloalkyl" and "cycloalkenyl" are defined as a cyclic C_{3-7} hydrocarbon group, e.g., cyclopropyl, cyclobutyl, cyclohexyl, cyclohexenyl, cyclopentenyl, and cyclopentyl. "Heterocycloalkyl" and "heterocycloalkenyl" are defined similarly as cycloalkyl except the ring contains one to three hereroatoms selected from the group consisting of oxygen, nitrogen, and sulphur. Cycloalkyl and heterocycloalkyl groups are saturated ring systems, and cycloalkenyl and heterocycloalkenyl are partially unsaturated ring systems, all optionally substituted with, for example, one to three groups, independently selected from C_{1-4}-alkyl, C_{1-3}-alkylene-OH, C_{1-3}alkylene-$N(R^a)_2$, NH_2, oxo (=O), aryl, and OH.

The term "halogen" is defined herein to include fluoro, bromo, chloro, and iodo.

The term"aryl", alone or in combination, is defined herein as a monocyclic or bicyclic aromatic group, e.g., phenyl or naphtyl. Unless otherwise indicated, an "aryl"group can be unsubstituted or substituted. Exemplary aryl groups include phenyl, naphthyl, tetrahydronaphthyl, chlorophenyl, methylphenyl, methoxyphenyl, trifluoromethylphenyl, nitrophenyl, 2,4-methoxychlorophenyl, and the like.

The term "heteroaryl" is defined herein as a monocyclic or bicyclic ring system containing at least one nitrogen, oxygen, or sulfur atom in an aromatic ring, and that can be unsubstituted or substituted. Examples of heteroaryl groups include, but are not limited to thienyl, furyl, pyridyl, oxazolyl, quinolyl, isoquinolyl, indolyl, triazolyl, isothiazolyl, isoxazolyl, imidizolyl, benzothiazolyl, pyrazinyl, pyrimidinyl, thiazolyl, and thiadiazolyl.

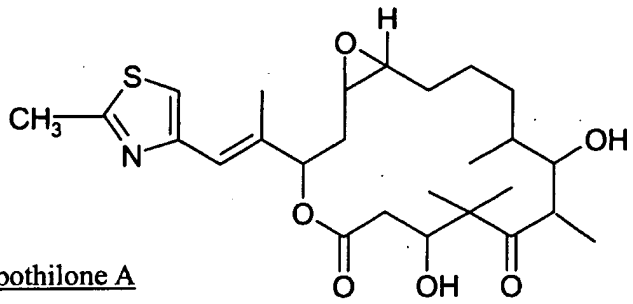
The term"alkoxy" is defined as —OR, wherein R is alkyl, including cycloalkyl.

The term "acyl" is defined as –CO-R, wherein R is alkyl, including cycloalkyl.

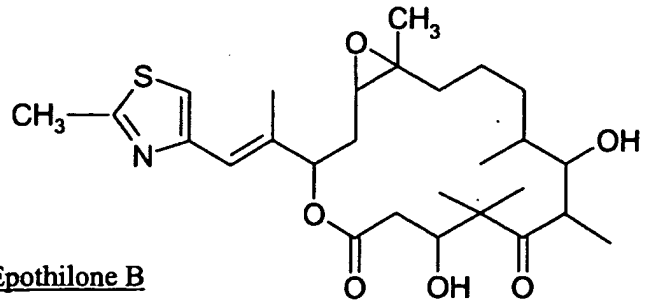
Epothilones of formula (I) and (II) are especially interesting as they appear to offer relief of both the positive and negative symptoms of schizophrenia. Further the present compounds also appear to be useful therapeutic agents for combating autism.

More particularly, the epothilone is at least a derivative of formula II wherein R'^1, R'^2, R'^3 represent independently from each other, H, C_1-C_6-alkyl in particular CH_3, C_1-C_6 perfluoroalkyl in particular CF_3 and Y and Z form together a C = C double bond or are together the O atom of an epoxide.

According to another specific embodiment, the used epothilones include at least the natural epothilones A or B of following formula:



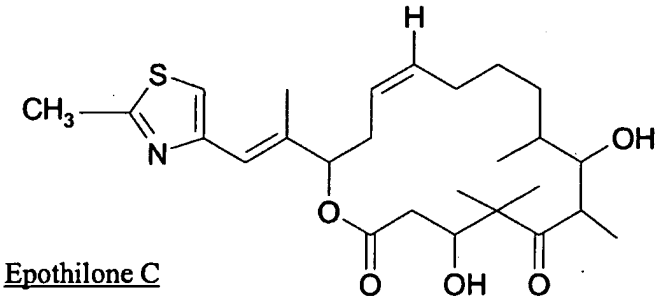
Epothilone A



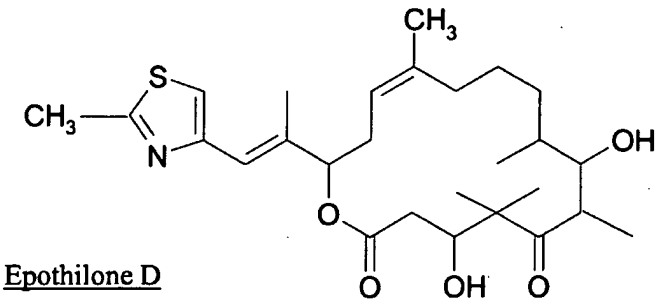
Epothilone B

and pharmaceutically acceptable salts thereof.

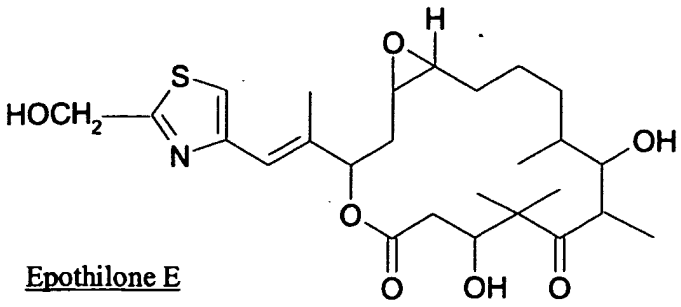
According to another specific embodiment, epothilones may be synthetic epothilone C, D, E or F of the following formula:



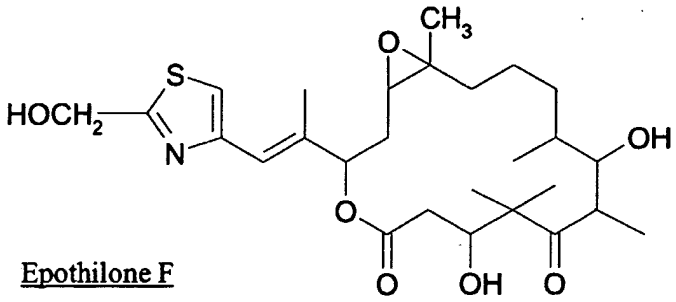
Epothilone C



Epothilone D



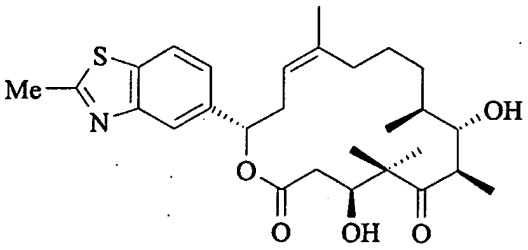
Epothilone E

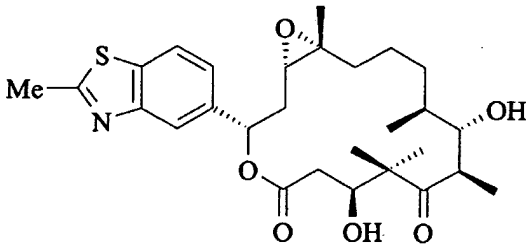


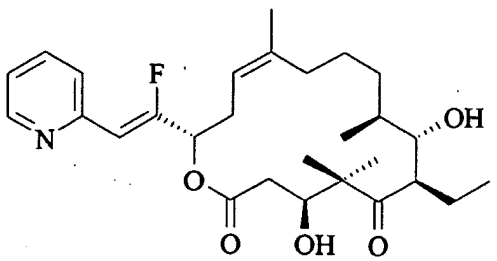
Epothilone F

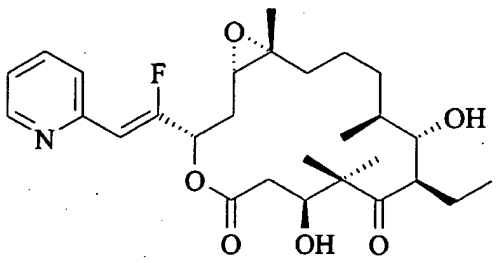
and in particular the epothilone D or derivative or salt thereof.

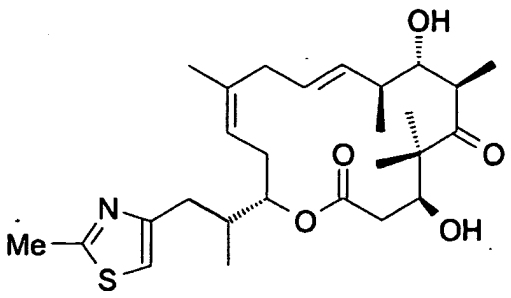
According to another specific embodiment, epothilone may be synthetic epothilone of following formula:

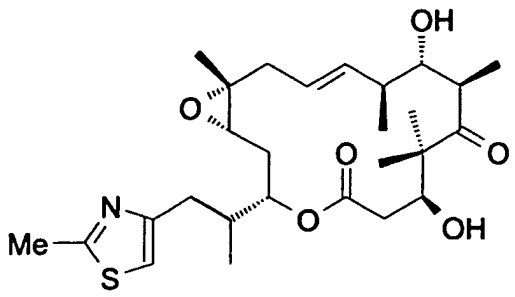












The term "therapeutically effective amount" as used herein refers to that amount in epothilone which, when administered to an individual in need thereof, is sufficient to provide efficient treatment, as defined below, for diseases associated with neuronal connectivity defect.

Naturally, the amount which constitutes a "therapeutically effective amount" will vary depending on the compound, the disease and its severity, and the age of the human to be treated, that can be determined routinely by one ordinary skill in the art having regard to his own knowledge and to this disclosure.

For example, the therapeutically effective amount in epothilone and in particular of a compound selected from the group consisting of Formula (I) or (II) may be from about 0.01 mg/Kg/dose to about 100 mg/Kg/dose. Preferably, the therapeutically effective amount may be from about 0.01 mg/Kg/dose to about 25 mg/Kg/dose. More preferably, the therapeutically effective amount may be from about 0.01 mg/Kg/dose to about 10 mg/Kg/dose. Most preferably, the therapeutically effective amount may be from about 0.01 mg/Kg/dose to about 5 mg/Kg/dose. Therefore, the therapeutically effective amount of the active ingredient contained per dosage unit (e.g., tablet, capsule, powder, injection, suppository, teaspoonful and the like) as described herein may be from about 1 mg/day to about 7000 mg/day for a subject, for example, having an average weight of 70 Kg.

For the use according to the invention, the epothilone can be formulated by methods known in the art. Compositions for the oral, rectal, parenteral or local application can be prepared in the form of tablets, capsules, granulates, suppositories, implantages, sterile injectable aqueous or oily solutions, suspensions or emulsions, aerosols, salves,

creams, or gels, retard preparations or retard implantates. The epothilone may also be administered by implantable dosing systems. In particular the epothilone is formulated for perfusion.

The pharmaceutical active epothilone can thus be mixed with adjuvants known in the art, such as gum Arabic, talcum, starch, mannitol, methyl cellulose, lactose, surfactants such as Tweens® or Myrj®, magnesium stearate, aqueous or non-aqueous carriers, paraffin derivatives, wetting agents, dispersing agents, emulsifiers, preservatives, and flavours.

An epothilone according to the invention or a pharmaceutical composition thereof may be administered by any conventional route of administration including, but not limited to oral, pulmonary, intraperitoneal (ip), intravenous (iv), intramuscular (im), subcutaneous (sc), transdermal, buccal, nasal, sublingual, ocular, rectal and vaginal. In addition, administration directly to the nervous system may include, and are not limited to, intracerebral, intraventricular, intracerebroventricular, intrathecal, intracisternal, intraspinal or peri-spinal routes of administration by delivery via intracranial or intravertebral needles or catheters with or without pump devices. It will be readily apparent to those skilled in the art that any dose or frequency of administration that provides the therapeutic effect described herein is suitable for use in the present invention.

The invention is further illustrated by the following examples and figures:

Figure 1:

It reports mouse activities treated or not with 3 mg/kg/week of epothilone D (sleeping, feeding, grooming, walking and remaining still while awake) that were video-recorded during 3 h. Upper panel (A) reports time spent in each different activity (calculated for each mouse and averaged). Lower panel (B) number of occurrences of each activity (calculated for each mouse and averaged).

*$p \leq 0.05$, ** $p \leq 0.01$, ***$p \leq 0.001$, ANOVA.

Figure 2:

It reports mouse activities (sleeping, feeding, grooming, walking and remaining still while awake) that were treated or not treated with epothilone D at a dose of 4

mg/kg/week. The activities are video-recorded during 3 h. Left Panels: number of occurrences of each activity (calculated for each mouse and averaged, mean ± s.e.m). Right Panels: time spent in each different activity (calculated for each mouse and averaged, mean ± s.e.m). Group sizes were: WT placebo: n=9; WT epothilone D: n=10; STOP KO placebo: n=10; STOP KO epothilone D: n=10

* p≤ 0.05, ** p≤ 0.025, *** p≤ 0.01, ANOVA.

Figure 3:

It reports nesting capacity of mice treated or not with a dose of epothilone D ranging from 0.3 mg/kg/week to 3 mg/kg/week. Tissue use score (T: 0-2), Nest building score (N: 0-2), Number of retrieved pups (R: 0-3= and global score (T+N+R) were calculated for each mouse and averaged (mean ± s.e.m.).

*p≤ 0.05, ** p≤ 0.01, ***p≤ 0.001, ANOVA.

Figure 4:

It reports the nesting capacity of mice treated or not with a dose of epothilone D at a dose of 4 mg/kg/week. Tissue use score (T: 0-2), Nest building score (N: 0-2), Number of retrieved pups (R: 1-3) and global score (T+N+R) were calculated for each mouse and averaged (mean ± s.e.m). Group sizes: as in figure 2.

p≤0.05, ** p≤ 0.025, *** p≤ 0.01, ANOVA

Figure 5:

It reports the quantitative analysis of synaptic vesicle density in CA1 hippocampal synapses of mice treated or not with epothilone D 3 mg/kg/week or with neuroleptics (0.5mg/kg/day haloperidol and 5mg/kg/day chlorpromazine, in drinking water, from birth to adulthood). Synaptic vesicle density, calculated as the ratio of the number of vesicle/nerve terminal surface (after subtraction of the surface area occupied by mitochondria). Results (means +/- s.e.m.) are shown for pooled 75 measurements from five wild type mice and from five STOP KO mice either untreated or treated with Epothilone D. For neuroleptic control experiments, results (means +/- s.e.m.) are shown for pooled 75 measurements from three STOP KO mice either untreated or treated with neuroleptics.

***p≤ 0.001, t-test.

Material and methods

Epothilone D has been tested in STOP deficient mice. These mice (STOP KO) display neuronal connectivity defects, with synaptic defects affecting both long- and – short term synaptic plasticity with a large depletion of synoptic vesicle pool within the hippocampal synapses (WT: 280 synaptic vesicles/μm^2 ; STOP KO: 150 synaptic vesicles/μm^2). The synaptic defects are associated with severe behavioral disorders. Behavioral disorders in STOP KO mice are alleviated by long-term treatment with neuroleptics. STOP KO mice are currently considered as a valuable animal model for study of the origin and treatment of mental diseases thought to result from a disease of the synapse, such as schizophrenia (Mirmics et al., Trends Neurosci., 2001, 24, 479-486).

Whereas behavioral defects in STOP KO mice are complex, these defects ultimately result in conspicuous alterations of spontaneous behaviour, with a fragmented activity characterized by frequent shifts between activities, and in severe deficits affecting tasks related to nurturing, such as nest building and pup retrieving . (Andrieux *et al.*, Genes Dev. 2002 Sep 15;16(18):2350-64). Both spontaneous activity and maternal behaviour were examined for test of epothilone effect.

Spontaneous activity (male and female) was recorded and quantified during a three hours period of time, as in Andrieux *et al.*, Genes Dev. 2002 Sep 15;16(18):2350-64. Five activities were considered: feeding, remaining still without sleeping, walking, grooming and sleeping. For each activity, the total time spent doing the activity and the number of distinct sequences of activity, were determined.

For maternal behaviour, STOP deficient or control wild type (WT) female mice, nulliparous, 8 weeks old, were treated either with a placebo (carrier alone), or with epothilone D.

The maternal behaviour of treated and untreated mice was monitored by assaying the mouse ability to build a nest and to retrieve pups

For assessment of nesting capacity, the tested mouse was placed in a 240x240x120 mm cage containing litter and provided with a Kleenex tissue folded in 4 (final dimensions, 100x100 mm). After 60 hours, the mouse ability to use the paper and to build a nest was scored as follows:

Tissue use score: 0, the Kleenex tissue remained folded; 1, the tissue has been unfolded but not shredded; 2 the tissue was shredded.

Nest building score: 0, no attempt to build a nest; 1, primitive flat nest of uncontrolled shape; 2, true nest, the paper is mixed with litter to form a circular nest, less than 80mm in diameter.

For assessment of retrieving: following the nest building test, mice were trained and assessed for pup retrieving as in Andrieux *et al.* (Genes Dev. 2002 Sep 15;16(18):2350-64. Retrieving was scored as the number of pups retrieved (0 to 3).

Finally a global score for nesting and retrieving capacity (TNR) was determined for each mouse, by adding the tissue use, nest building, and pup retrieving scores (maximal score for TNR is 7).

The effect of epothilones treatment was also analyzed on the pool of synaptic vesicles.

Hippocampus was dissected out from transcardially fixed mice, sliced and embedded in Epon. To determine the surface density of synaptic vesicles, a total of cross sections of 75 synapses made of hippocampal CA1 region were photographed randomly, and the numbers of synaptic vesicles in each nerve pre-terminal were counted on the electron micrographs. Synaptic vesicles density of STOP KO mice following long term neuroleptic treatment (0.5mg/kg/day haloperidol and 5mg/kg/day chlorpromazine, in drinking water, from birth to adulthood) was also examined using the same protocol.

In a first series of experiments, epothilone D was injected intra-peritoneally, once a week, at either 0.3 mg/kg/week or 3 mg/kg/week for at least 8 weeks. The drug was diluted at a final concentration of 0.3 mg/ml in water, from a 50 mg/ml stock solution in DMSO.

In a second series of experiments, epothilone D was injected intra-peritoneally, in two injections a week, at a total dose of 4 mg/kg/week, for 8 weeks. The drug was diluted at a final concentration of 0.2mg/ml in water, from a 50mg/ml stock solution in DMSO.

Example I:

Effect of epothilone D on spontaneous activity

Among 61 STOP KO mice, 28 were treated with placebo injections, 33 with 3mg/kg/week of epothilone D. Among 62 WT mice, 29 were treated with placebo injections, 33 with 3mg/kg/week of epothilone D.

In STOP deficient mice, epothilone D treatment with 3 mg/kg/week caused a decrease in the total time spent grooming and an increase in the total time spent sleeping (figure 1-A). Epothilone D treatment also decreased the total number of shifts between activities (figure 1-B), reducing both the number of walking and remaining still while awake sequences.. These results obtained on a large number of animals including both males and females indicate an alleviation of the activity fragmentation that is characteristic of untreated STOP KO mice (Andrieux *et al.*, Genes Dev. 2002 Sep 15;16(18):2350-64).

In another series of experiments, among 19 STOP KO mice, 9 were treated with placebo injections, 10 with 4mg/kg/week epothilone D. Among 20 WT mice, 10 were treated with placebo injections and 10 with 4 mg/kg/week epothilone D.

In STOP deficient mice, epothilone D treatment with 4mg/kg/week for 8 weeks caused a remarkable decrease in the total number of shifts between activities (see Figure 2). This decrease in number concerned the number of walking, and grooming sequences, whereas the number of sleeping and feeding sequences remained unaffected. The total time spent grooming was also highly significantly diminished by epothilone treatment. Finally, epothilone treatment tended to diminish the time spent remaining still without sleeping, and to increase the time spent sleeping. Again, these results indicate a conspicuous alleviation of the activity fragmentation that is characteristic of untreated STOP deficient mice (Andrieux *et al.*, Genes Dev. 2002 Sep 15;16(18):2350-64), with a trend to diminish abnormal activities such as remaining still without sleeping, or activities that can be stereotypic such as grooming, and to increase sleeping which is deficient in untreated STOP KO mice (Andrieux *et al.*, Genes Dev. 2002 Sep 15;16(18):2350-64).

Example II:

Effect of epothilone D on maternal behaviour

The maternal behaviour of untreated and treated mice was monitored by assaying mouse ability to build a nest and to retrieve pups. Experiments were performed at 3 concentrations of epothilone D (4 mg/kg/week, 3 mg/kg/week and 0.3 mg/kg/week).

Among 69 STO KO mice, 2 groups of 17 were treated with placebo injections, 17 were treated with 3 mg/kg/week of epothilone, and 18 were treated with 0.3 mg/kg/week of epothilone. Among 34 WT mice, 17 were treated with placebo injections, and 17 were treated with 3 mg/kg/week of épothilone.

Epothilone treatment at both 3 mg/kg/week and 0.3 mg/kg/week improved the paper use score, the nest building score, and the pup retrieving score inducing a highly significant increase in the TNR score (Figure 3). Epothilone treatment at both 3 mg/kg/week and 0.3 mg/kg/week thus had a remarkable beneficial effect on nurturing-related tasks that are strongly deficient in untreated STOP KO mice.

The epothilone treatment has been then tested whether it could induce pup survival in STOP KO mothers. Nine STOP KO mice treated with Epothilone D at 0.3 mg/kg/week and eight untreated females were mated the pups survival were analysed after delivery. Remarkably, in three out of the nine STOP KO females subjected to epothilone D treatment, nurturing improvement was sufficient to permit pup survival with ratios of surviving pups to newborns of 5/6, 6/6, 5/6, for the three mice. Accordingly with previous observations (Andrieux *et al.*, Genes Dev. 2002 Sep 15;16(18):2350-64) in the absence of treatment, pup survival did not occur in the progeny from STOP KO mothers. Pups survival observed after epothilone D treatment can be compared to the occurrence of pup survival in long-term neuroleptic treated STOP KO mice where the pups survival were observed in four out of seven STOP KO females (ratios of surviving pups to newborns of 3/11, 4/8, 2/4, 1/5, for the four mice). These results indicate a remarkable capacity of epothilone D in re-establishing maternal abilities compatible with pup survival in STOP KO mice.

In another series of experiments, among 19 STOP KO mice, 9 were treated with placebo injections, 10 with 4mg/kg/week epothilone D and as control, among 20 WT mice, 10 were treated with placebo injections and 10 with 4 mg/kg/week epothilone D.

In STOP deficient mice, epothilone treatment at 4 mg/kg/week strongly improved the paper use score, the nest building score, and tended to improve the pup retrieving score (Figure 4). There was a highly significant increase in the TNR score, which rose from 1.16 to 4.4, upon epothilone treatment, in STOP KO mice (Figure 4). The treatment thus had a remarkable beneficial effect on nurturing-related tasks that are strongly deficient in untreated STOP KO mice.

In both example I and example II, epothilone treatment had no significant effect on the recorded activities or on nurturing-related behaviours in WT mice, with the exception of a significant increase in the number of sequences of stillness in example II (Figures 2). However, the observation of a single significant difference among multiple comparisons is compatible with random fluctuations. Altogether, results indicate that epothilones have little or no psychotropic effects in WT mice.

Taken together, these results show that epothilone treatment can alleviate behavioural disorders in an animal model of psychiatric disease involving connectivity disorders while unaffecting WT mice.

Example III:

Effect of epothilones an hippocampal synaptic vesicle density

For this study hippocampal sections from 5 WT and 5 STOP KO mice either untreated or treated with epothilone D at 4 mg/kg/week were used.

After epothilone D treatment, the synaptic vesicle density increased in STOP KO mice (Figure 5). This increase represented 20% of the vesicle density observed in the absence of treatment and was highly significant ($p<0.001$). This increase of the vesicle number after epothilone treatment was within the same range as the one observed in parallel experiments using hippocampal sections from 3 STOP KO mice

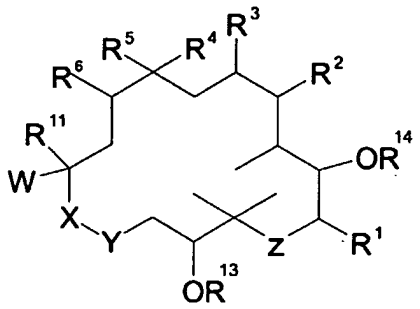
either untreated or treated with neuroleptics (Figure 5).

CLAIMS

1. Use of at least one epothilone or derivative thereof as an active ingredient for manufacturing a medicament for use in the treatment of disease(s) involving a neuronal connectivity defect.

2. Use of at least one epothilone or derivative thereof as an active ingredient for manufacturing a medicament for use in the treatment of schizophrenia or autism.

3. Use according to claim 1 or 2, wherein the epothilone is a compound of formula (I):



(I)

wherein:

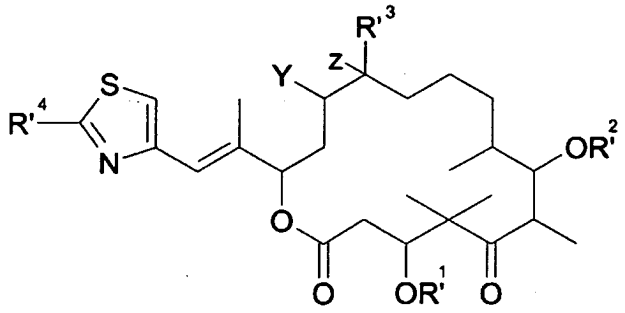
- R^1 represents H, alkyl, alkenyl or alkynyl in C_1-C_6, aryl in C_6-C_{10}, aralkyl in C_7-C_{15},
- R^2, R^3 represents each H or form together C=C double bond,
- R^4 represents H, C_1-C_6-alkyl in particular CH_3, fluoro substituted C_1-C_6 alkyl in particular CF_3 or CFH_2,
- R^5 and R^6 form a C=C double bond or a three membered ring including O, S, NR^7, CR^8R^9 with R^7 being $C(O)R^{10}$, SO_2R^{10} and R^8, R^9, R^{10} being independently H, halogen, C_1-C_6 alkyl, C_6-C_{10} aryl, C_7-C_{15} alkaryl,
- R^{11} being H, C_1-C_6 alkyl, C_6-C_{10} aryl, C_7-C_{15} alkaryl, and in particular H,
- W represents $C(R^{12})$=CH, $C(R^{12})$=$C(CH_3)$, $C(R^{12})$=CF or a bicyclic aromatic/heteroaromatic radical preferably a 2-methylbenzothiazol-5-yl radical, or a 2-methylbenzoxazol-5-yl radical or a quinolin-7-yl radical, with R^{12} representing a heteroaromatic radical, preferably a 2-pyridinyl, a 2-substituted thiazol-4-yl or a 2-substituted oxazol-4-yl radical with substitution in 2-position by C_1-C_6 alkyl,

pseudohalogen like CN or N_3, S-C_1-C_4-alkyl, O-C_1-C_6-alkyl, or C_1-C_6-alkyl substituted by OH, amino, halogen, pseudohalogen such as –NCO, -NCS, $–N_3$, O-(C_1-C_6)-acyl, O-(C_1-C_6)-alkyl or O-benzoyl,

- X-Y represents O-C(=O), O-CH_2, CH_2-O, CH_2-C(=O),
- Z represents C=O, S, S=O, SO_2,
- R^{13} and R^{14} represents independently from each other H, C_1-C_6-alkyl, (CO)R^{15} or C_{1-4}-trialkylsilyl, with R^{15} being H, C_1-C_6-alkyl, fluoro substituted C_1-C_6-alkyl,

and pharmaceutically acceptable salts thereof.

4. Use according to any one of claims 1 to 3, wherein the epothilone is a derivative of following formula (II):



(II)

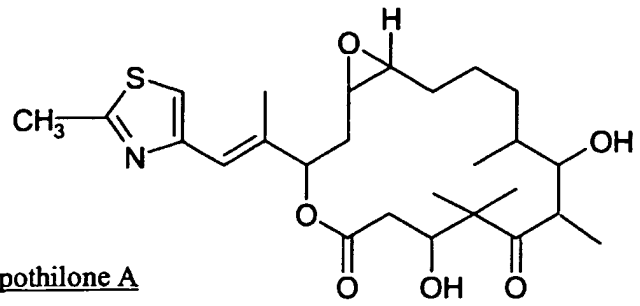
wherein:

- R'^{4} represents an C_1-C_6 alkyl or substituted C_1-C_6 alkyl with substituents as F, Cl, Br or I, pseudohalogen, such as –NCO, -NCS , $–N_3$, NH_2, OH, O-(C_1-C_6)-acyl, O-(C_1-C_6)-alkyl or O-benzoyl,
- R'^{1} and R'^{2} are independently from each other H, C_1-C_6-alkyl, (CO)R'^{5} with R'^{5} being H, C_1-C_6-alkyl, C_1-C_6-fluoroalkyl or C_{1-4}-trialkylsilyl,
- R'^{3} represents H, C_1-C_6-alkyl, halogen substituted C_1-C_6-alkyl, and
- Y and Z form either a C=C double bond or are the O atom of an epoxide

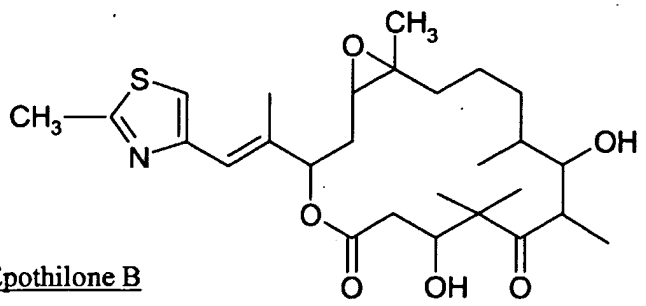
and pharmaceutically acceptable salts thereof.

5. Use according to claim 4, wherein the epothilone is at least a derivative of formula (II) wherein R'^{1}, R'^{2}, R'^{3} represents independently from each other, H, C_1-C_6-alkyl in particular CH_3, C_1-C_6 fluoroalkyl in particular CF_3 and Y and Z form either a C=C double bond or are together the O atom of an epoxide.

6. Use according to any one of claims 1 to 5, wherein epothilone includes at least the natural epothilone A or B of following formula:



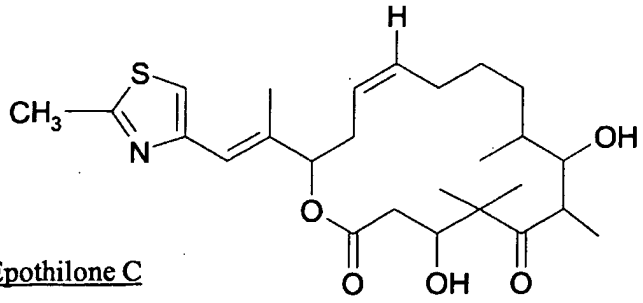
Epothilone A



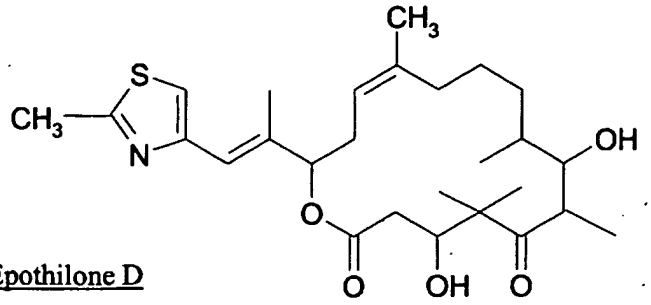
Epothilone B

or a pharmaceutically acceptable salt thereof.

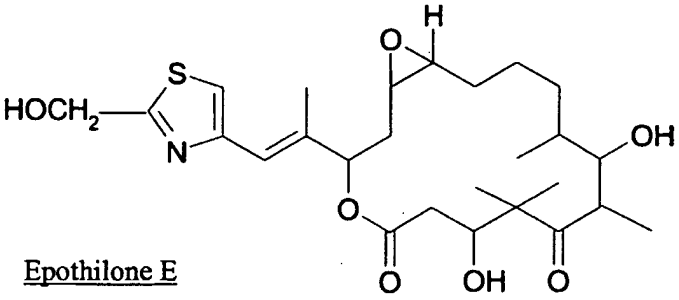
7. Use according to any one of claims 1 to 6, wherein epothilone includes at least one synthetic epothilone C, D, E or F of following formula:



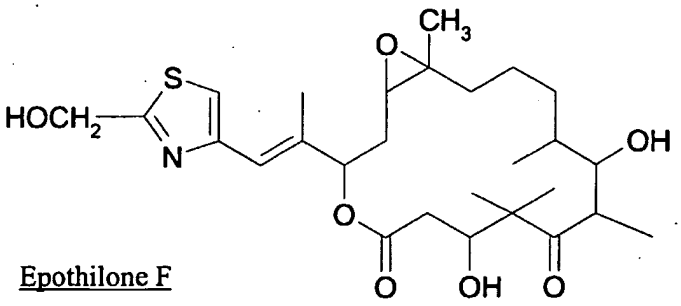
Epothilone C



Epothilone D



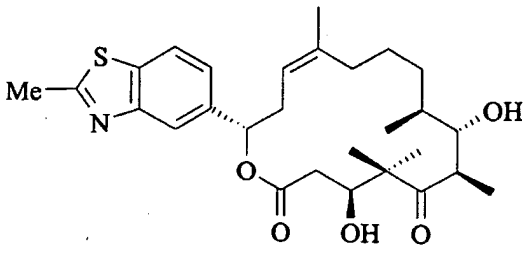
Epothilone E

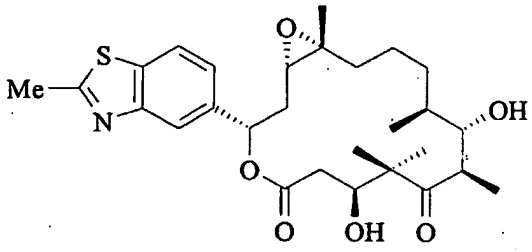


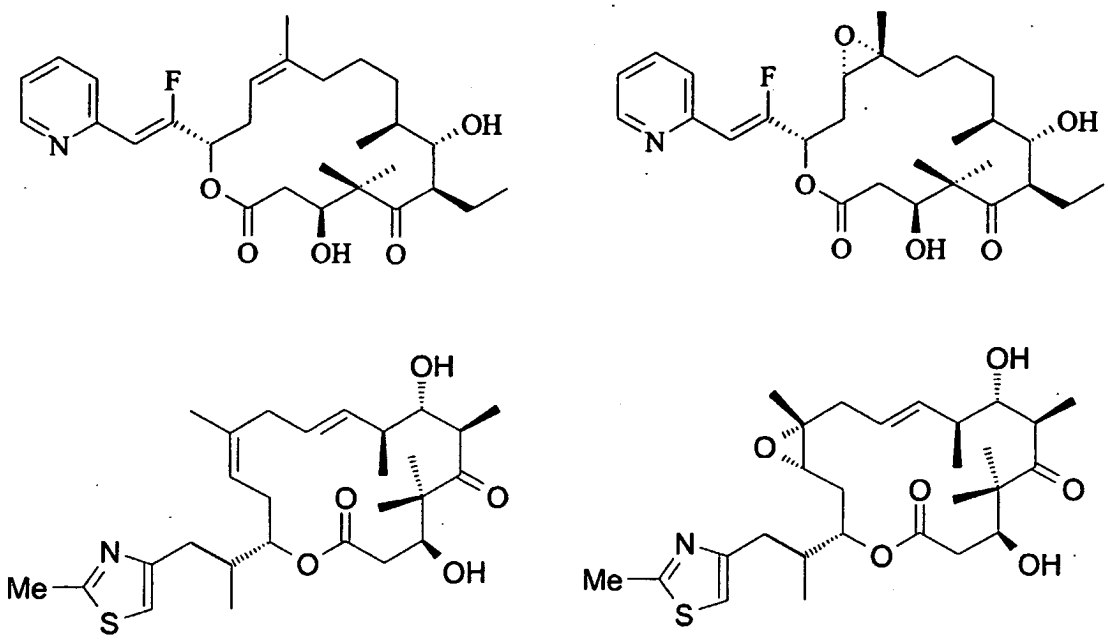
Epothilone F

in particular epothilone D and pharmaceutically acceptable salts thereof.

8. Use according to any one of claims 1 to 7, wherein epothilone includes at least one synthetic epothilone of following formula:







9. Use according to any one of claims 1 to 8, wherein the epothilone(s) is used at a therapeutically effective amount from about 0.01/Kg/dose to about 100 mg/Kg/dose.

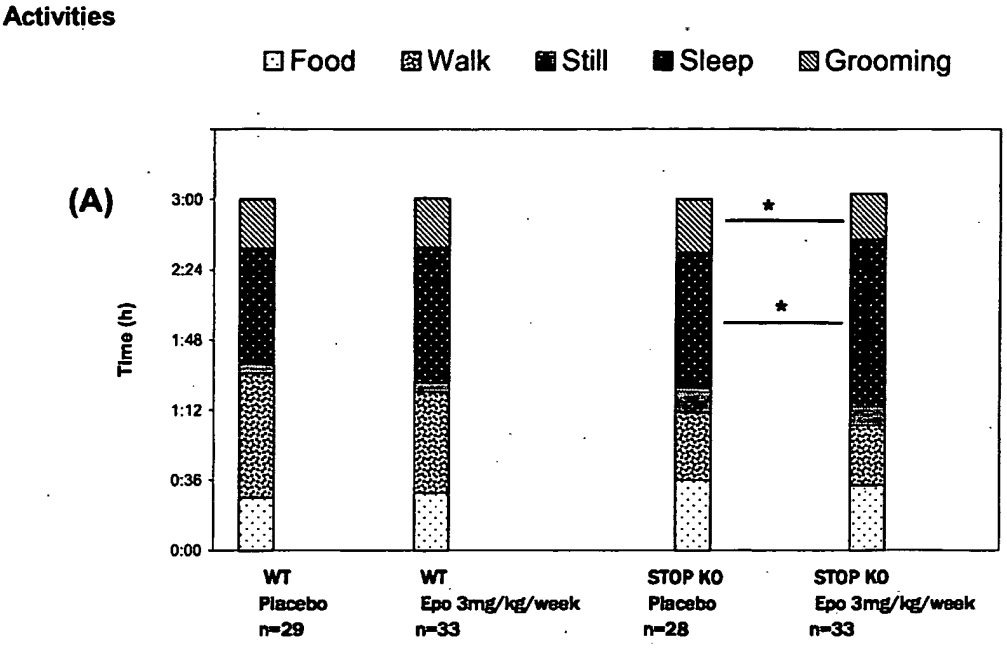
10. Method of treatment of a disease involving a neuronal connectivity defect comprising administering to an individual in need thereof a therapeutic effective amount of one epothilone or derivative thereof.

11. Method of treatment of a disease involving a neuronal connectivity defect comprising administering to an individual a therapeutically effective amount of at least one epothilone or derivative thereof in a pharmaceutical composition comprising at least a pharmaceutically acceptable carrier.

12. Method according to claim 10 or 11, wherein the disease includes a psychotic or psychiatric disorder.

13. Method according to any one of claims from 10 to 12, wherein the epothilone or pharmaceutical compositions thereof is administered in combination with one or more agents useful in preventing or treating psychotic or psychiatric disorders.

14. Method according to any one of claims from 10 to 13, wherein the epothilone is as defined in claims 3 to 9.


Activities
Food
Walk
Still
Sleep
Grooming
(A)
Time (h)
3:00
2:24
1:48
1:12
0:36
0:00
*
*
WT Placebo n=29
WT Epo 3mg/kg/week n=33
STOP KO Placebo n=28
STOP KO Epo 3mg/kg/week n=33

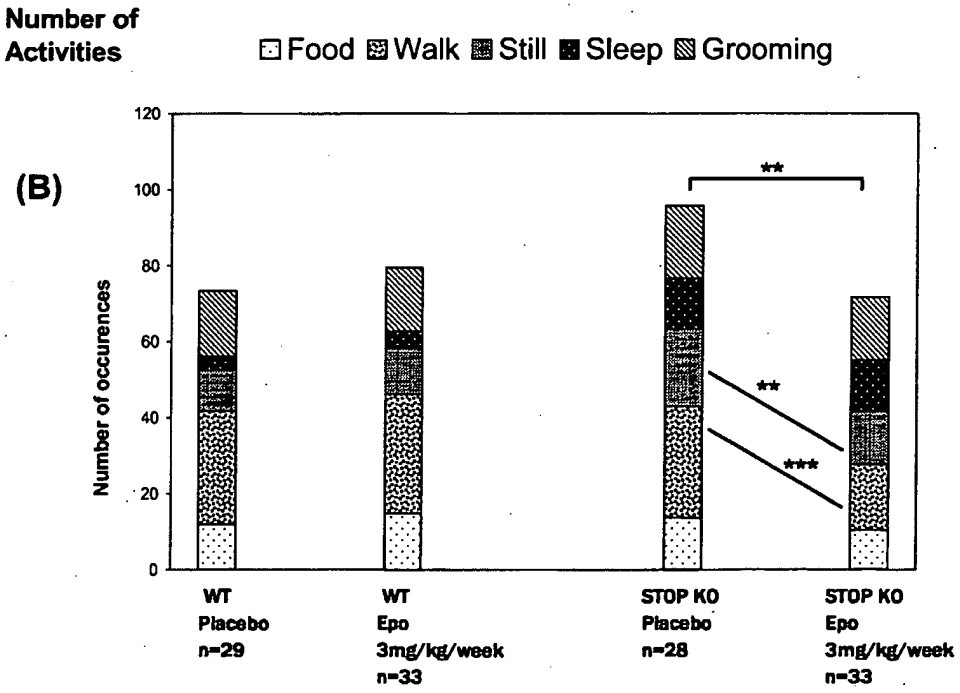

Number of Activities
Food
Walk
Still
Sleep
Grooming
(B)
Number of occurences
120
100
80
60
40
20
0
**
**
***
WT Placebo n=29
WT Epo 3mg/kg/week n=33
STOP KO Placebo n=28
STOP KO Epo 3mg/kg/week n=33

FIGURE 1

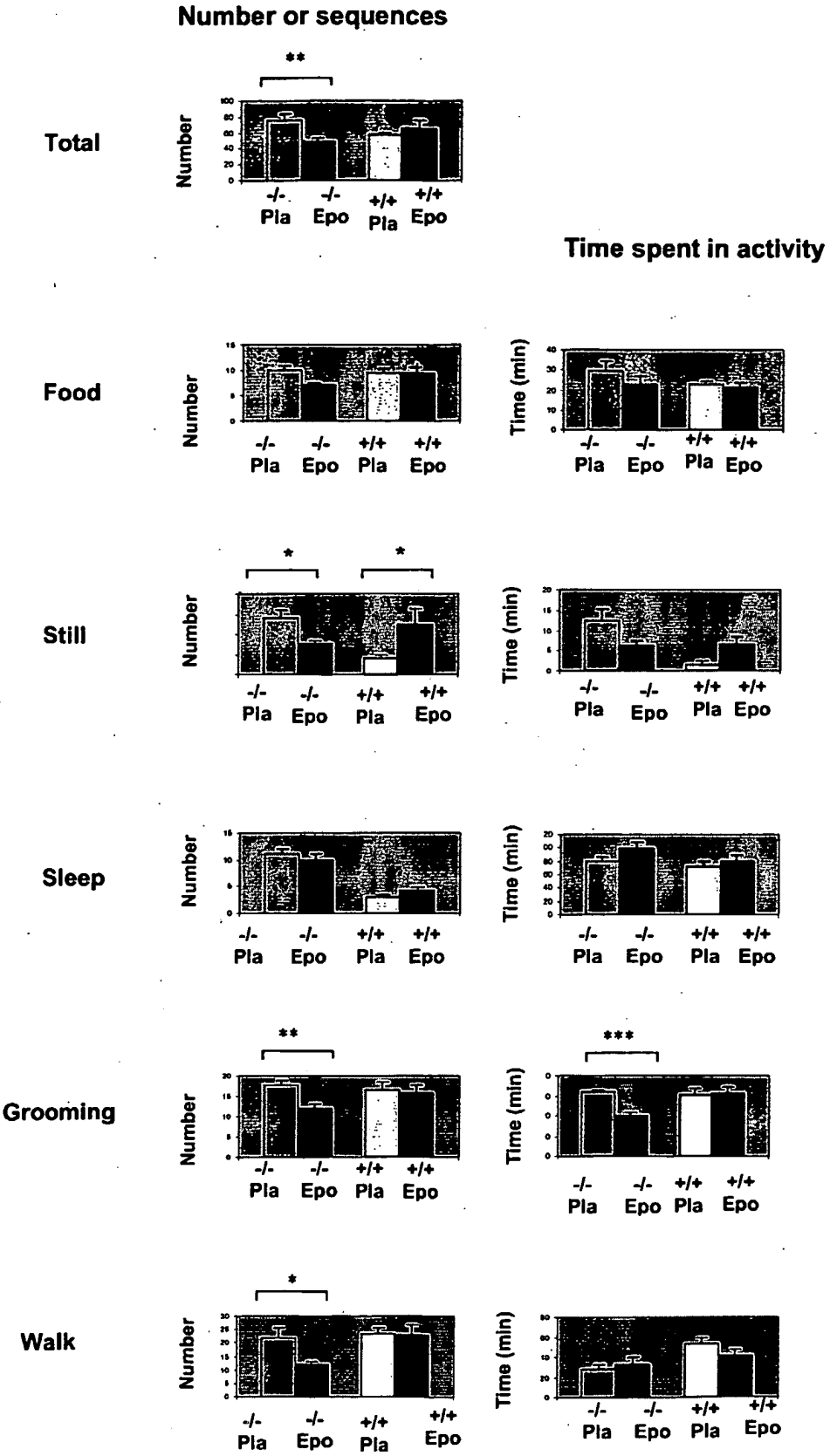

Number or sequences
Time spent in activity
Total
Food
Still
Sleep
Grooming
Walk
Number
Time (min)
**
*
*
**
***
*
-/- Pla
-/- Epo
+/+ Pla
+/+ Epo

FIGURE 2

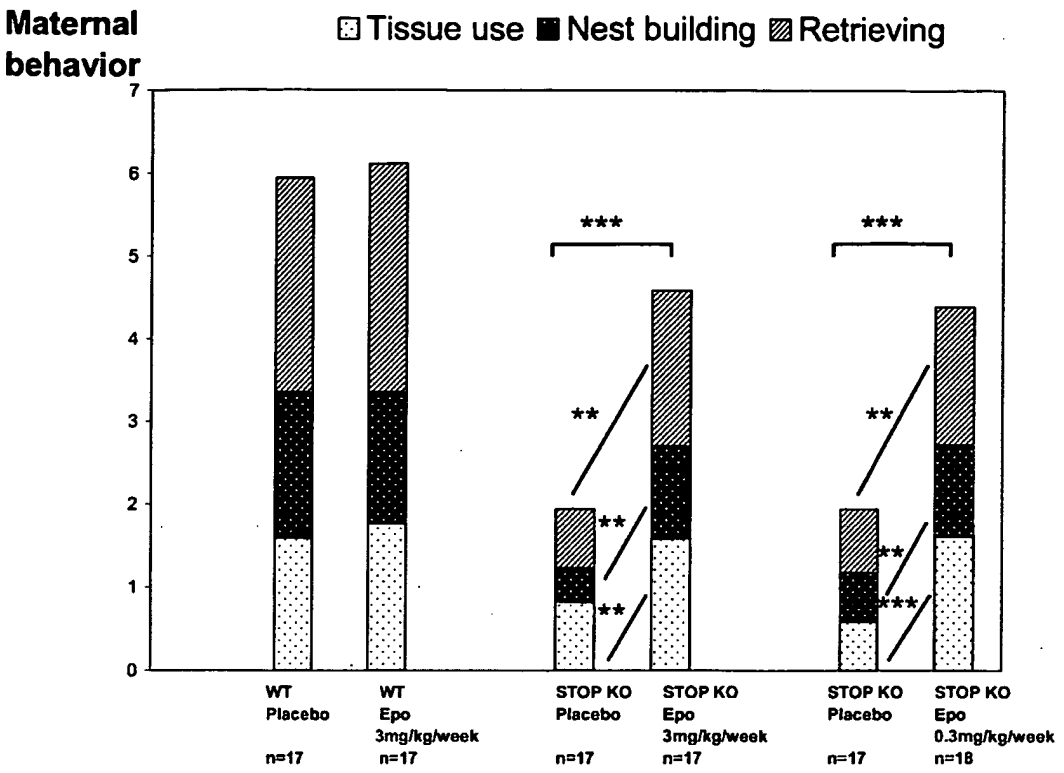

Maternal behavior
Tissue use
Nest building
Retrieving
7
6
5
4
3
2
1
0
***
***
**
**
**
**
**
***
WT Placebo n=17
WT Epo 3mg/kg/week n=17
STOP KO Placebo n=17
STOP KO Epo 3mg/kg/week n=17
STOP KO Placebo n=17
STOP KO Epo 0.3mg/kg/week n=18

FIGURE 3

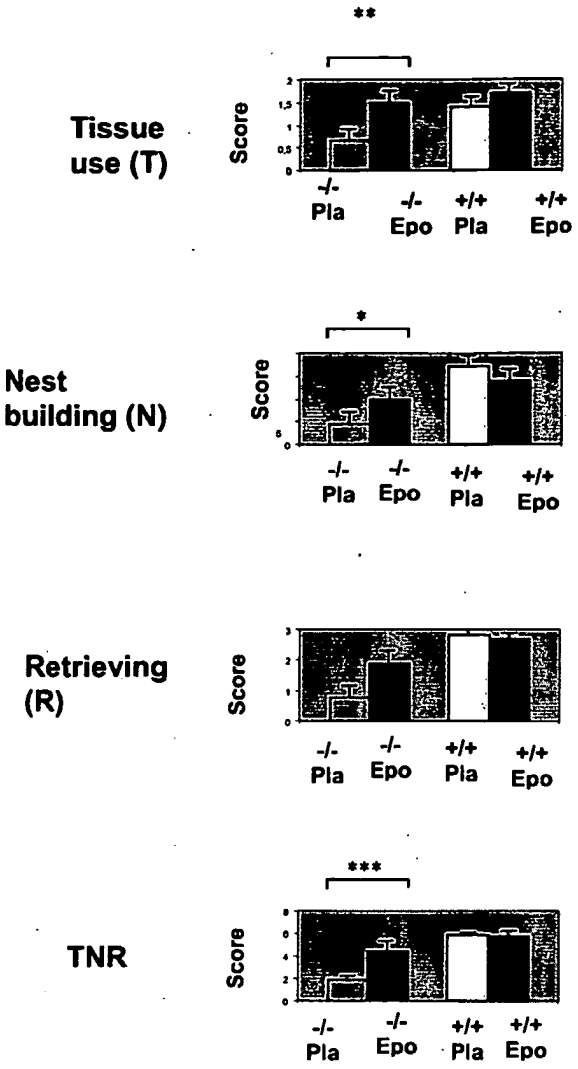

**
Tissue use (T)
Score
-/- Pla
-/- Epo
+/+ Pla
+/+ Epo
*
Nest building (N)
Score
-/- Pla
-/- Epo
+/+ Pla
+/+ Epo
Retrieving (R)
Score
-/- Pla
-/- Epo
+/+ Pla
+/+ Epo
***
TNR
Score
-/- Pla
-/- Epo
+/+ Pla
+/+ Epo

FIGURE 4

0

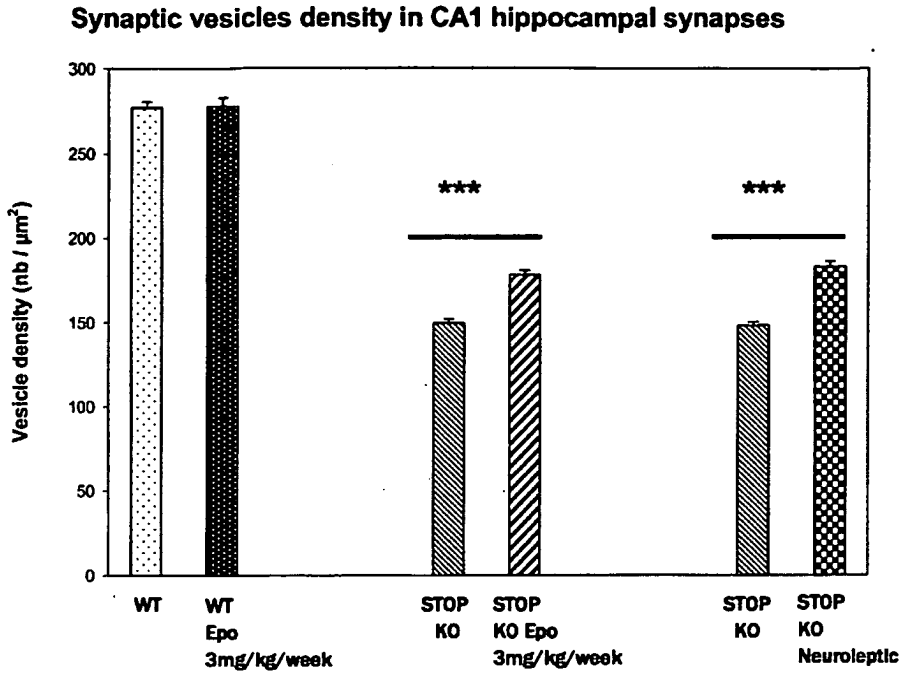

Synaptic vesicles density in CA1 hippocampal synapses
Vesicle density (nb / µm²)
300
250
200
150
100
50
0
***
***
WT
WT Epo 3mg/kg/week
STOP KO
STOP KO Epo 3mg/kg/week
STOP KO
STOP KO Neuroleptic

FIGURE 5

INTERNATIONAL SEARCH REPORT

International Application No
/IB2005/000217

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61P25/18 A61K31/335 A61K31/38 A61K31/425

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, BIOSIS, CHEM ABS Data, PASCAL, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 03/074053 A (HOFFMANN JENS ;KLAR ULRICH (DE); SCHERING AG (DE); BUCHMANN BERND) 12 September 2003 (2003-09-12) cited in the application page 1, lines 6-8	1-14
A	US 6 518 421 B1 (SWAMINATHAN SHANKAR ET AL) 11 February 2003 (2003-02-11) column 7, line 37; examples 2-4,7,8	1-14
A	NICOLAOU K C ET AL: "Chemical Biology of Epothilones" ANGEWANDTE CHEMIE. INTERNATIONAL EDITION, VERLAG CHEMIE. WEINHEIM, DE, vol. 37, no. 15, August 1998 (1998-08), pages 2014-2045, XP002131418 ISSN: 0570-0833 abstract	1-14

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 April 2005	04/05/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Allnutt, S

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2005/000217

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 10-14
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 10-14 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
/IB2005/000217

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 03074053 A	12-09-2003	EP 1340498 A1	03-09-2003
		AU 2003215618 A1	16-09-2003
		BR 0308154 A	04-01-2005
		CA 2477403 A1	12-09-2003
		WO 03074053 A1	12-09-2003
		EP 1480643 A1	01-12-2004
		HR 20040892 A2	31-12-2004
		US 2004019088 A1	29-01-2004
US 6518421 B1	11-02-2003	WO 02060904 A2	08-08-2002
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		CA 2404212 A1	27-09-2001
		EP 1265878 A1	18-12-2002
		HU 0300693 A2	28-08-2003
		JP 2003528090 T	24-09-2003
		WO 0170716 A1	27-09-2001

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92*bis*.1 and Administrative Instructions, Section 422)

To:

LE COUPANEC, Pascale
Nony & Partners
3 rue de Penthièvre
F-75008 Paris
FRANCE

Date of mailing *(day/month/year)* 12 September 2006 (12.09.2006)	
Applicant's or agent's file reference BR78668/DC1	IMPORTANT NOTIFICATION
International application No. PCT/IB2005/000217	International filing date *(day/month/year)* 28 January 2005 (28.01.2005)

1. The following indications appeared on record concerning:

☒ the applicant ☐ the inventor ☐ the agent ☐ the common representative

Name and Address	State of Nationality	State of Residence
GESELLSCHAFT FUR BIOTECHNOLOGISCHE FORSCHUNG MGH (GBF) Mascheroder Weg 1 38124 Braunschweig Germany	DE	DE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address	State of Nationality	State of Residence
GESELLSCHAFT FUR BIOTECHNOLOGISCHE FORSCHUNG MBH (GBF) Mascheroder Weg 1 38124 Braunschweig Germany	DE	DE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☐ the receiving Office
☐ the International Searching Authority
☐ the International Preliminary Examining Authority
☒ the designated Offices concerned
☐ the elected Offices concerned
☐ other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Pouvreau Pascalle Facsimile No. +41 22 338 70 60 Telephone No. +41 22 338 95 46

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92*bis*.1 and Administrative Instructions, Section 422)

To:

LE COUPANEC, Pascale
Nony & Partners
3 rue de Penthièvre
F-75008 Paris
FRANCE

Date of mailing *(day/month/year)*	
12 September 2006 (12.09.2006)	
Applicant's or agent's file reference BR78668/DC1	IMPORTANT NOTIFICATION
International application No. PCT/IB2005/000217	International filing date *(day/month/year)* 28 January 2005 (28.01.2005)

1. The following indications appeared on record concerning:

☒ the applicant ☒ the inventor ☐ the agent ☐ the common representative

Name and Address	State of Nationality	State of Residence
HÖFLE, Gerhard Alter Weg 12a 38124 Branschweig Germany	DE	DE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address	State of Nationality	State of Residence
HÖFLE, Gerhard Alter Weg 12a 38124 Braunschweig Germany	DE	DE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☐ the receiving Office
☐ the International Searching Authority
☐ the International Preliminary Examining Authority
☒ the designated Offices concerned
☐ the elected Offices concerned
☐ other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Pouvreau Pascalle
Facsimile No. +41 22 338 82 70	Facsimile No. +41 22 338 70 60 Telephone No. +41 22 338 95 46

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92*bis*.1 and Administrative Instructions, Section 422)

To:

LE COUPANEC, Pascale
Nony & Partners
3 rue de Penthièvre
F-75008 Paris
FRANCE

Date of mailing *(day/month/year)*
12 September 2006 (12.09.2006)

Applicant's or agent's file reference
BR78668/DC1

IMPORTANT NOTIFICATION

International application No.
PCT/IB2005/000217

International filing date *(day/month/year)*
28 January 2005 (28.01.2005)

1. The following indications appeared on record concerning:

☒ the applicant ☒ the inventor ☐ the agent ☐ the common representative

Name and Address
HÖFLE, Gerhard
Alter Weg 12a
38124 Branschweig
Germany

State of Nationality: DE
State of Residence: DE
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address
HÖFLE, Gerhard
Alter Weg 12a
38124 Braunschweig
Germany

State of Nationality: DE
State of Residence: DE
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☐ the receiving Office
☐ the International Searching Authority
☐ the International Preliminary Examining Authority
☒ the designated Offices concerned
☐ the elected Offices concerned
☐ other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. +41 22 338 82 70

Authorized officer
Pouvreau Pascalle
Facsimile No. +41 22 338 70 60
Telephone No. +41 22 338 95 46

PATENT COOPERATION TREATY

PCT

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Applicant's or agent's file reference see form PCT/ISA/220	Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet) **FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB2005/000217	28.01.2005	30.01.2004

International Patent Classification (IPC) or both national classification and IPC
A61P25/18, A61K31/335, A61K31/38, A61K31/425

Applicant
INSTITUT NATIONAL DE LA SANTE ET DE LA...

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Allnutt, S

Telephone No. +49 89 2399-7817

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IB2005/000217

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 10-14

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 10-14 (Industrial Applicability)

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form ☐ has not been furnished

☐ does not comply with the standard

the computer readable form ☐ has not been furnished

☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-14
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-14
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	
	No:	Claims	10-14

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Claims 10-14 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

2.The documents cited in the International Search Report are consecutively numbered D1-D3 in this communication;this numbering will be adhered to in the rest of the procedure. The cited passage(s) for each citation will be considered unless otherwise specified.

Novelty and Inventive Step (Articles 33(2) and (3) PCT)

3. The technical features of claims 1-14 are not anticipated by documents D1-3 and therefore fulfill the criteria of novelty and inventive step in terms of Art 33 (2) and (3) PCT.

D1 discloses the use of epothilones in the treatment of brain diseases associated with proliferative processes. It does not mention neuronal connectivity defects or treatment of e.g. schizophrenia or psychiatric disorders.
D2 discloses the use of epothilone derivatives for numerous disorders including AIDS-related dementia. However no diseases associated with neuronal connectivity disorders are discussed.
D3 is a general review document on epothilones.

Epothilones are known in the prior art as anticancer agents, however there is no hint or disclosure from prior art documents D1-D3 that they are able to treat diseases such as schizophrenia, autism, psychotic disorders or other diseases related neuronal connectivity disorders.
The application also provides evidence that epothilone D appears to alleviate behavioural disorders in an animal model of psychiatric disease.

Industrial Applicability (Article 33(4) PCT)

4. For the assessment of the present claims 10-14 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VIII

Certain observations on the international application

5. Present claims 1,10 and 11 relate to the treatment of a disease which is not well defined. The use of the definition "neuronal connectivity defect" in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is impossible to determine the diseases for which protection might legitimately be sought.

6. Formula I of claim 3, defines W as C(R12)=C**H**, however its dependent claims 4-8 and specific compounds disclosed therein have a substituent where W is infact C(R12)=C**CH3** i.e. it is not defined within the scope of claim 3 thus leading to a lack of clarity (Art 6 PCT).

7. Claims 4-7 do not fall under the scope of claim 3 upon which they are partially dependent therefore leading to a lack of clarity (Art 6 PCT).
Formula I of claim 3 does not define R4 as H, whereas the compounds in claims 6 and 7 have this definition as well as formula II in claim 4.

8. Claim 13 does not meet the requirements of Article 84 EPC in that the matter for which protection is sought is not clearly defined. The active agent is defined functionally, namely "useful in preventing or treating psychotic or psychiatric disorders" which does not enable the skilled person to determine which exact compounds are encompassed by this term.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference BR78668/DC1	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/IB2005/000217	International filing date *(day/month/year)* 28 January 2005 (28.01.2005)	Priority date *(day/month/year)* 30 January 2004 (30.01.2004)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE (INSERM)		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 31 July 2006 (31.07.2006)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Cecile Chatel e-mail: pt13@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

PCT

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Applicant's or agent's file reference see form PCT/ISA/220	Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet) **FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB2005/000217	28.01.2005	30.01.2004

International Patent Classification (IPC) or both national classification and IPC
A61P25/18, A61K31/335, A61K31/38, A61K31/425

Applicant
INSTITUT NATIONAL DE LA SANTE ET DE LA...

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Allnutt, S

Telephone No. +49 89 2399-7817

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IB2005/000217

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 10-14

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 10-14 (Industrial Applicability)

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form ☐ has not been furnished

☐ does not comply with the standard

the computer readable form ☐ has not been furnished

☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-14
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-14
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	
	No:	Claims	10-14

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Claims 10-14 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

2.The documents cited in the International Search Report are consecutively numbered D1-D3 in this communication;this numbering will be adhered to in the rest of the procedure. The cited passage(s) for each citation will be considered unless otherwise specified.

Novelty and Inventive Step (Articles 33(2) and (3) PCT)

3. The technical features of claims 1-14 are not anticipated by documents D1-3 and therefore fulfill the criteria of novelty and inventive step in terms of Art 33 (2) and (3) PCT.

D1 discloses the use of epothilones in the treatment of brain diseases associated with proliferative processes. It does not mention neuronal connectivity defects or treatment of e.g. schizophrenia or psychiatric disorders.
D2 discloses the use of epothilone derivatives for numerous disorders including AIDS-related dementia. However no diseases associated with neuronal connectivity disorders are discussed.
D3 is a general review document on epothilones.

Epothilones are known in the prior art as anticancer agents, however there is no hint or disclosure from prior art documents D1-D3 that they are able to treat diseases such as schizophrenia, autism, psychotic disorders or other diseases related neuronal connectivity disorders.
The application also provides evidence that epothilone D appears to alleviate behavioural disorders in an animal model of psychiatric disease.

Industrial Applicability (Article 33(4) PCT)

4. For the assessment of the present claims 10-14 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VIII

Certain observations on the international application

5. Present claims 1,10 and 11 relate to the treatment of a disease which is not well defined. The use of the definition "neuronal connectivity defect" in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is impossible to determine the diseases for which protection might legitimately be sought.

6. Formula I of claim 3, defines W as C(R12)=C**H**, however its dependent claims 4-8 and specific compounds disclosed therein have a substituent where W is infact C(R12)=C**CH3** i.e. it is not defined within the scope of claim 3 thus leading to a lack of clarity (Art 6 PCT).

7. Claims 4-7 do not fall under the scope of claim 3 upon which they are partially dependent therefore leading to a lack of clarity (Art 6 PCT).
Formula I of claim 3 does not define R4 as H, whereas the compounds in claims 6 and 7 have this definition as well as formula II in claim 4.

8. Claim 13 does not meet the requirements of Article 84 EPC in that the matter for which protection is sought is not clearly defined. The active agent is defined functionally, namely "useful in preventing or treating psychotic or psychiatric disorders" which does not enable the skilled person to determine which exact compounds are encompassed by this term.

PCT/IB05/217

Europäisches Patentamt | European Patent Office | Office européen des brevets

Bescheinigung | Certificate | Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

04290249.4

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

Anmeldung Nr:
Application no.: 04290249.4
Demande no:

Anmeldetag:
Date of filing: 30.01.04
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

INSTITUT NATIONAL DE LA SANTE ET DE LA
RECHERCHE MEDICALE (INSERM)
101, rue de Tolbiac
75654 Paris Cédex 13
FRANCE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Use of epothilones in the treatment of psychotic disorders with neuronal connectivity defects

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

A61K31/00

Am Anmeldetag benannte Vertragstaaten/Contracting states designated at date of filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL
PT RO SE SI SK TR LI

Use of epothilones in the treatment of psychotic disorders with neuronal connectivity defects

The present invention relates to the use of epothilones for manufacturing a medicament for use in the treatment of psychotic disorders thought to be associated with neuronal connectivity defects, in the absence of obvious anatomical, degenerative, or proliferative anomalies.

Psychotic disorders are disorders that are predominantly characterized by an impairment of mental functioning to the extent that it interferes grossly with an individual's ability to meet the ordinary demands of life.

Psychotic disorders currently thought to result from disorders in neuronal connectivity include, and are not limited to, schizophrenia, schizophreniform disorder, schizoaffective or delusional disorder, and autism (Andreassen NC, Brain Res. Rev. 2000; 31:106-12; Francke *et al.*, Neuron, 2003, 39, 205-216; Jamain *et al*, Nature Genetics, 2003, 34, 27-28.l).

Schizophrenia is any of a group of psychotic disorders usually characterized by withdrawal from reality, illogical patterns of thinking, delusions and hallucinations and accompanied in varying degrees by other emotional, behavioral or intellectual disturbances. A lifelong chronic mental illness, schizophrenia exhibits positive and negative symptoms, with an onset in young adulthood and deterioration from the previous level of functioning. Positive symptoms reflect a distortion or excess of normal functions (eg, disorganized speech, delusions, and hallucinations). Negative symptoms, on the other hand, reflect a restricted range of normal behavior and emotions (eg, apathy, paucity of speech and incongruity or flattening of emotional responses). Schizophrenia can be presented in various forms depending on the symptoms and signs. The varieties of schizophrenia include paranoid schizophrenia, hebephrenic schizophrenia, catatonic schizophrenia, and undifferentiated schizophrenia as well as post-schizophrenic depression, residual schizophrenia, simple schizophrenia and unspecified schizophrenia.

Neuroleptic agents or drugs (i.e. "neuroleptics"), also called anti-psychotics or major tranquilizers remain the treatment of choice for schizophrenia, certain organic

central nervous system disorders, mental illnesses, and associated psychotic processes today. Currently, it is estimated that over 95% of schizophrenics are chronically maintained on neuroleptics. All known neuroleptics are dopaminergic blockers, having pharmacological actions similar to the action of chlorpromazine, an aliphatic phenothiazine derivative also known as Thiorazine.

Although the known anti-psychotic drugs have clear efficacy in the treatment of mental illness, they do not cure all symptoms and have a variety of side effects.

The invention derives from the discovery that epothilones can alleviate schizophrenia-related behavioural disorders in an animal model termed STOP deficient mice (Andrieux et al.: Genes Dev. 2002 Sep 15; 16(18): 2350-64).

The natural products Epothilones A and B as well as some of their synthetic derivatives have recently found interest in connection with the treatment of cancer. Thus, WO 98/22461, WO 99/07692, DE 198 21954, WO 99/02514, WO 99/67252, WO 00/50423, WO 02/21712, WO 00/66589, WO 01/081341, WO 00/49021 and US 2003/0 203 929 deal with the synthesis of epothilone derivatives and for some of them with their use in the treatment of cancers. WO 03/074053 relates more specifically to the use of some epothilone derivatives to treat diseases involving degenerative or hyperproliferative processes, such as brain tumor, Alzeihmer's disease, multiple sclerosis and primary or secondary brain tumors.

A beneficial effect of epothilones has now been demonstrated in STOP deficient mice which display neuronal connectivity defects not associated with any detectable anatomical, degenerative or proliferative anomalies.

Accordingly, the invention provides alternative therapies for treating some Central Nervous System (CNS) disorders and mental illness, associated with neuronal connectivity defect, particularly schizophrenia and autism, said defects being not associated with any detectable anatomical, degenerative or proliferative anomalies.

According to a first aspect, the present invention is directed to the use of at least one epothilone or derivative thereof as an active ingredient, in particular in a

therapeutically effective amount, for manufacturing a medicament for use in the treatment of disease(s) involving a neuronal connectivity defect.

In particular, the present invention is directed to the use of at least one epothilone or derivative thereof as an active ingredient, in particular in a therapeutically effective amount, for manufacturing a medicament for use in the treatment of schizophrenia and/or autism.

According to a second aspect, the present invention is directed to a method of treatment of disease(s) involving a neuronal connectivity defect comprising administering to an individual in need thereof a therapeutically effective amount of at least one epothilone or derivative thereof, in particular as defined according to the invention.

An embodiment of the method of the present invention comprises administering to an individual a therapeutically effective amount of at least one epothilone or derivative thereof, in particular as defined according to the instant invention, in a pharmaceutical composition comprising at least a pharmaceutically acceptable carrier.

Another embodiment of the method of the present invention comprises administering to an individual a therapeutically effective amount of at least one epothilone or derivative thereof, in particular according to the invention or a pharmaceutical composition thereof in combination with one or more agents useful in preventing or treating psychotic or psychiatric disorders, in particular such as neuroleptics.

As used herein, the term "disease associated with neuronal connectivity defect" refers to mental diseases currently thought to involve neuronal connectivity disorder, in the absence of obvious anatomical, proliferative or degenerative anomaly. Examples of such disorders include particularly schizophrenia and autism. In particular, the diseases considered according to the invention are different from progressive dementing disorders like Alzheimer, which involve neuronal degeneration.

As used herein, the term "schizophrenia" refers to a psychiatric disorder that includes at least two of the following: delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behaviour, or negative symptoms. Patients can be

diagnosed as schizophrenic using the DSM-IV criteria (APA, 1994, *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition), Washington, D.C.).

"Negative" symptoms of schizophrenia include affect blunting, anergia, alogia and social withdrawal, which can be measured using SANS (the Scales for the Assessment of Negative Symptoms; see Andreasen, 1983, *Scales for the Assessment of Negative Symptoms* (SANS), Iowa City, Iowa).

"Positive" symptoms of schizophrenia include delusion and hallucination, which can be measured using PANSS (the Positive and Negative Syndrome Scale; see Kay et al., 1987, Schizophrenia Bulletin 13:261-276).

"Cognitive" symptoms of schizophrenia include impairment in obtaining, organizing, and using intellectual knowledge which can be measured by the Positive and Negative Syndrome Scale-cognitive subscale (PANSS-cognitive subscale) (Lindenmayer et al., 1994, J. Nerv. Ment. Dis. 182:631-638) or with cognitive tasks such as the Wisconsin Card Sorting Test.

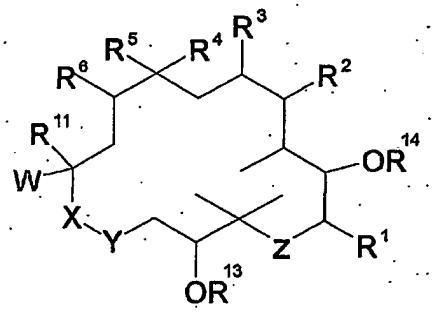
As used herein, the term "autism" refers to a state of mental introversion characterized by morbid self-absorption, social failure, language delay, and stereotyped behavior. Patients can be diagnosed as suffering from autism by using the DSM-IV criteria.

"Treating" or "treatment" as used herein refers to the treatment of a disease in an individual, which disease is associated with neuronal connectivity defect and includes:

(i) inhibiting the disease, i.e., arresting its development; or

(ii) relieving the disease, i.e., alleviating symptoms caused by the disease.

According to one embodiment, the used epothilone may be derivatives of epothilones of following formula (I):



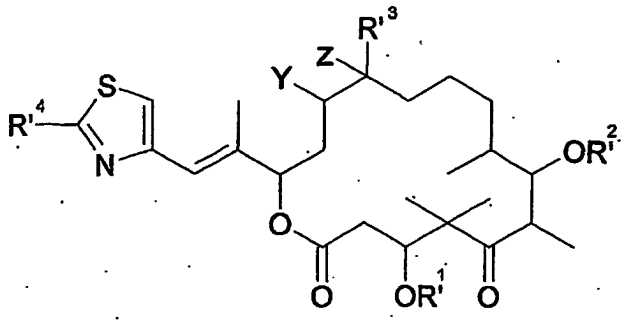
(I)

wherein:

- R^1 represents H, alkyl, alkenyl or alkynyl in C_1-C_6, aryl in C_6-C_{10}, aralkyl in C_7-C_{15},
- R^2, R^3 represents each H or form together C=C double bond,
- R^4 represents C_1-C_6-alkyl in particular CH_3, fluoro substituted C_1-C_6 alkyl in particular CF_3 or CFH_2,
- R^5 and R^6 form a C=C double bond or a three membered ring including O, S, NR^7, CR^8R^9 with R^7 being $C(O)R^{10}$, SO_2R^{10} and R^8, R^9, R^{10} being independently H, halogen, C_1-C_6 alkyl, C_6-C_{10} aryl, C_7-C_{15} alkaryl,
- R^{11} being H, C_1-C_6 alkyl, C_6-C_{10} aryl, C_7-C_{15} alkaryl, and in particular H,
- W represents $C(R^{12})$=CH, $C(R^{12})$=CF or a bicyclic aromatic/heteroaromatic radical preferably a 2-methylbenzothiazol-5-yl radical, or a 2-methylbenzoxazol-5-yl radical or a quinolin-7-yl radical, with R^{12} representing a heteroaromatic radical, preferably a 2-pyridinyl, a 2-substituted thiazol-4-yl or a 2-substituted oxazol-4-yl radical with substitution in 2-position by C_1-C_6-alkyl, pseudohalogen like CN or N_3, S-C_1-C_4-alkyl, O-C_1-C_6-alkyl, or C_1-C_6-alkyl substituted by OH, amino, halogen, pseudohalogen such as –NCO, -NCS, $–N_3$, O-(C_1-C_6)-acyl, O-(C_1-C_6)-alkyl or O-benzoyl,
- X-Y represents O-C(=O), O-CH_2, CH_2-O, CH_2-C(=O),
- Z represents C=O, S, S=O, SO_2,
- R^{13} and R^{14} represents independently from each other H, C_1-C_6-alkyl, $(CO)R^{15}$ or C_{1-4}-trialkylsilyl, with R^{15} being H, C_1-C_6-alkyl, fluoro substituted C_1-C_6-alkyl, and

pharmaceutically acceptable salts thereof.

According to one embodiment, the used epothilone may be derivatives of epothilones of following formula (II):



(II)

wherein:

- R'^4 represents an C_1-C_6-alkyl or substituted C_1-C_6-alkyl with substituents as F, Cl, Br or I, pseudohalogen such as –NCO, -NCS, $–N_3$, NH_2, OH, O-(C_1-C_6)-acyl, O-(C_1-C_6)-alkyl or O-benzoyl,
- R'^1 and R'^2 are independently from each other H, C_1-C_6-alkyl, $(CO)R'^5$ with R'^5 being H, C_1-C_6-alkyl, C_1-C_6-fluoroalkyl or C_{1-4}-trialkylsilyl,
- R'^3 represents H, C_1-C_6-alkyl, halogen substituted C_1-C_6-alkyl, and
- Y and Z form either a C=C double bond or are the O atom of an epoxide

and pharmaceutically acceptable salts thereof.

Depending on the nature of the various subsistuents, the compounds of formula (I) and (II) may have several asymmetric carbon atoms. Unless otherwise mentioned or indicated, the chemical designation of compounds denotes the mixture of all possible stereochemically isomeric forms, said mixtures containing all diastereomers and enantiomers of the basic molecular structure.

The term addition salt as used hereinabove also comprises the solvates which the compounds of formula (I) and (II) are able to form and said solvates are meant to be included within the scope of the present invention. Examples of such solvates are e.g., the hydrates, alcoholates and the like.

As used therein,

the term "alkyl" includes straight chained and branched hydrocarbon groups containing the indicated number of carbon atoms, typically methyl, ethyl, and straight-chain and branched propyl and butyl groups. Unless otherwise indicated, the hydrocarbon group can obtain up to 16 carbon atoms. The term "alkyl" includes "bridged alkyl". Alkyl

groups can be substituted, for example, with hydroxy (OH), halogen, aryl, heteroaryl, cycloalkyl, heterocycloalkyl, cycloalkenyl, heterocycloalkenyl, amino ($N(R^b)_2$), and sulfonyl (SO_2R^b), wherein R^b is selected from the group consisting of hydro, C_1-C_6alkyl, cycloalkyl, aryl, and SO_2C_1-C_6-alkyl, or two R^b groups are taken together to form a 5- or 6-membered ring.

The terms "cycloalkyl" and "cycloalkenyl" are defined as a cyclic C_{3-7} hydrocarbon group, e.g., cyclopropyl, cyclobutyl, cyclohexyl, cyclohexenyl, cyclopentenyl, and cyclopentyl. "Heterocycloalkyl" and "heterocycloalkenyl" are defined similarly as cycloalkyl except the ring contains one to three hereroatoms selected from the group consisting of oxygen, nitrogen, and sulphur. Cycloalkyl and heterocycloalkyl groups are saturated ring systems, and cycloalkenyl and heterocycloalkenyl are partially unsaturated ring systems, all optionally substituted with, for example, one to three groups, independently selected from C_{1-4}-alkyl, C_{1-3}-alkylene-OH, C_{1-3}alkylene-$N(R^a)_2$, NH_2, oxo (=O), aryl, and OH.

The term "halogen" is defined herein to include fluoro, bromo, chloro, and iodo.

The term"aryl", alone or in combination, is defined herein as a monocyclic or bicyclic aromatic group, e.g., phenyl or naphtyl. Unless otherwise indicated, an "aryl"group can be unsubstituted or substituted. Exemplary aryl groups include phenyl, naphthyl, tetrahydronaphthyl, chlorophenyl, methylphenyl, methoxyphenyl, trifluoromethylphenyl, nitrophenyl, 2,4-methoxychlorophenyl, and the like.

The term "heteroaryl" is defined herein as a monocyclic or bicyclic ring system containing at least one nitrogen, oxygen, or sulfur atom in an aromatic ring, and that can be unsubstituted or substituted. Examples of heteroaryl groups include, but are not limited to thienyl, furyl, pyridyl, oxazolyl, quinolyl, isoquinolyl, indolyl, triazolyl, isothiazolyl, isoxazolyl, imidizolyl, benzothiazolyl, pyrazinyl, pyrimidinyl, thiazolyl, and thiadiazolyl.

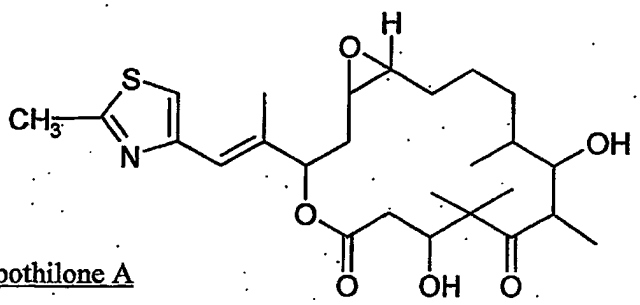
The term"alkoxy" is defined as —OR, wherein R is alkyl, including cycloalkyl.

The term "acyl" is defined as –CO-R, wherein R is alkyl, including cycloalkyl.

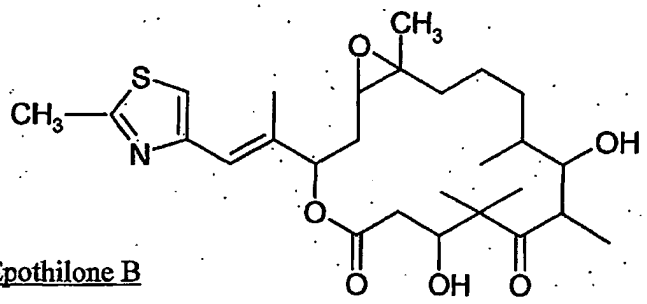
Epothilones of formula (I) and (II) are especially interesting as they appear to offer relief of both the positive and negative symptoms of schizophrenia. Further the present compounds also appear to be useful therapeutic agents for combating autism.

More particularly, the epothilone is at least a derivative of formula II wherein R'^1, R'^2, R'^3 represent independently from each other, H, C_1-C_6-alkyl in particular CH_3, C_1-C_6 perfluoroalkyl in particular CF_3 and Y and Z form together a C = C double bond or are together the O atom of an epoxide.

According to another specific embodiment, the used epothilones include at least the natural epothilones A or B of following formula:



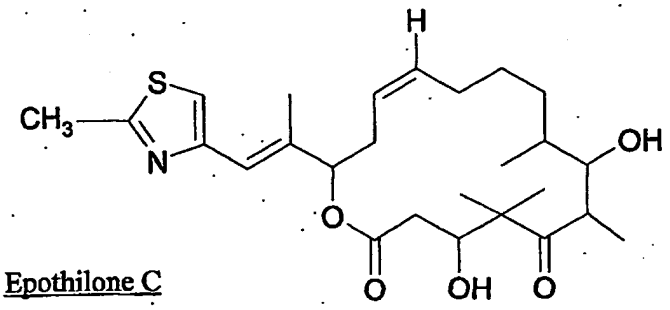
Epothilone A



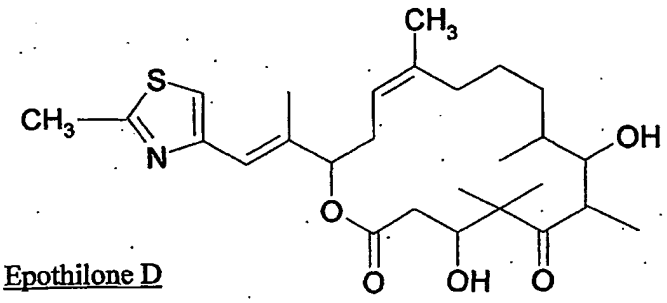
Epothilone B

and pharmaceutically acceptable salts thereof.

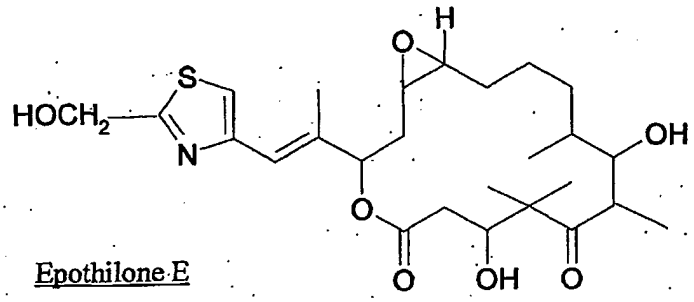
According to another specific embodiment, epothilones may be synthetic epothilone C, D, E or F of the following formula:



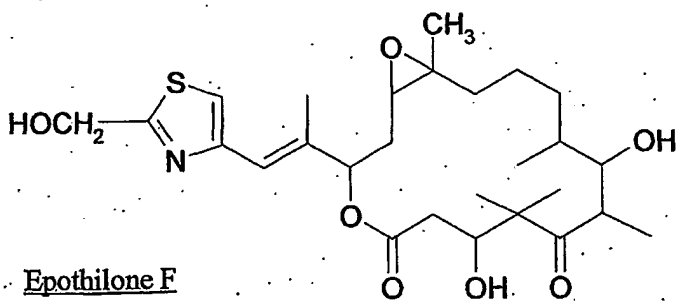
Epothilone C



Epothilone D



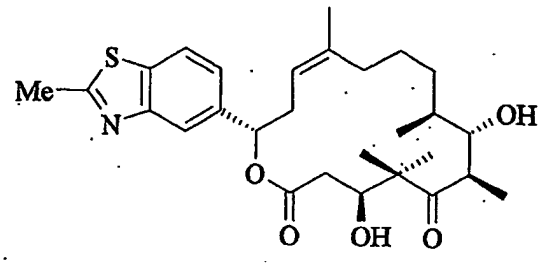
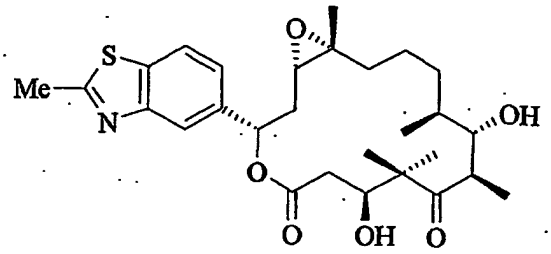
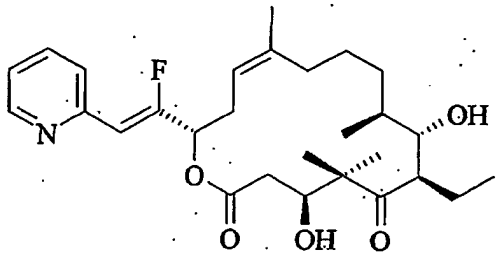
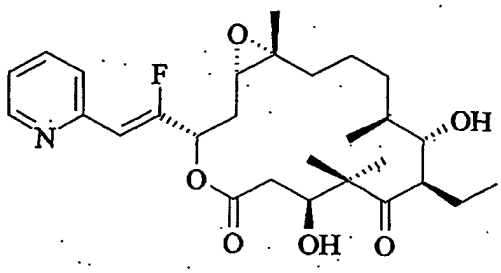
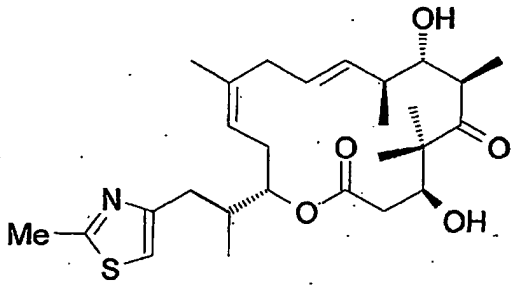
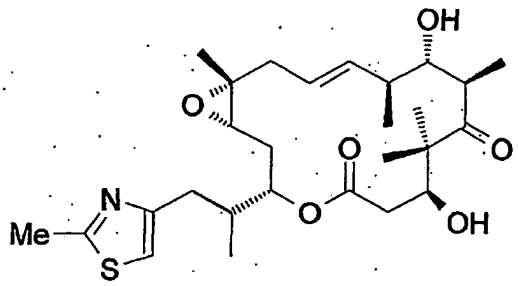
Epothilone E



Epothilone F

and in particular the epothilone D or derivative or salt thereof.

According to another specific embodiment, epothilone may be synthetic epothilone of following formula:

The term "therapeutically effective amount" as used herein refers to that amount in epothilone which, when administered to an individual in need thereof, is sufficient to provide efficient treatment, as defined below, for diseases associated with neuronal connectivity defect.

Naturally, the amount which constitutes a "therapeutically effective amount" will vary depending on the compound, the disease and its severity, and the age of the human to be treated, that can be determined routinely by one ordinary skill in the art having regard to his own knowledge and to this disclosure.

For example, the therapeutically effective amount in epothilone and in particular of a compound selected from the group consisting of Formula (I) or (II) may be from about 0.01 mg/Kg/dose to about 100 mg/Kg/dose. Preferably, the therapeutically effective amount may be from about 0.01 mg/Kg/dose to about 25 mg/Kg/dose. More preferably, the therapeutically effective amount may be from about 0.01 mg/Kg/dose to about 10 mg/Kg/dose. Most preferably, the therapeutically effective amount may be from about 0.01 mg/Kg/dose to about 5 mg/Kg/dose. Therefore, the therapeutically effective amount of the active ingredient contained per dosage unit (e.g., tablet, capsule, powder, injection, suppository, teaspoonful and the like) as described herein may be from about 1 mg/day to about 7000 mg/day for a subject, for example, having an average weight of 70 Kg.

For the use according to the invention, the epothilone can be formulated by methods known in the art. Compositions for the oral, rectal, parenteral or local application can be prepared in the form of tablets, capsules, granulates, suppositories, implantages, sterile injectable aqueous or oily solutions, suspensions or emulsions, aerosols, salves, creams, or gels, retard preparations or retard implantates. The epothilone may also be administered by implantable dosing systems. In particular the epothilone is formulated for perfusion.

The pharmaceutical active epothilone can thus be mixed with adjuvants known in the art, such as gum Arabic, talcum, starch, mannitol, methyl cellulose, lactose, surfactants such as Tweens® or Myrj®, magnesium stearate, aqueous or non-aqueous carriers, paraffin derivatives, wetting agents, dispersing agents, emulsifiers, preservatives, and flavours.

An epothilone according to the invention or a pharmaceutical composition thereof may be administered by any conventional route of administration including, but not limited to oral, pulmonary, intraperitoneal (ip), intravenous (iv), intramuscular (im), subcutaneous (sc), transdermal, buccal, nasal, sublingual, ocular, rectal and vaginal. In addition, administration directly to the nervous system may include, and are not limited to, intracerebral, intraventricular, intracerebroventricular, intrathecal, intracisternal, intraspinal or peri-spinal routes of administration by delivery via intracranial or intravertebral needles or catheters with or without pump devices. It will be readily apparent to those skilled in the

art that any dose or frequency of administration that provides the therapeutic effect described herein is suitable for use in the present invention.

The invention is further illustrated by the following examples and figures:

Figure 1:

It reports mouse activities (sleeping, feeding, grooming, walking and remaining still while awake) treated or not treated with epothilone D. The activities are video-recorded during 3 h. Left Panels: number of occurrences of each activity (calculated for each mouse and averaged, mean ± s.e.m). Right Panels: time spent in each different activity (calculated for each mouse and averaged, mean ± s.e.m).

* $p \leq 0.05$, ** $p \leq 0.025$, *** $p \leq 0.01$, ANOVA.

Figure 2:

It reports the nesting capacity. Tissue use score (T: 0-2), Nest building score (N: 0-2), Number of retrieved pups (R: 1-3) and global score (T+N+R) were calculated for each mouse and averaged (mean ± s.e.m).

$p \leq 0.05$, ** $p \leq 0.025$, *** $p \leq 0.01$, ANOVA

Example 1

Epothilone D has been tested in STOP deficient mice. These mice display neuronal connectivity defects, with synaptic defects affecting both long- and – short term synaptic plasticity, associated with severe behavioral disorders. Behavioral disorders in STOP-/- mice are alleviated by long-term treatment with neuroleptics. STOP-/- mice are currently considered as a valuable animal model for study of the origin and treatment of mental diseases thought to result from a disease of the synapse, such as schizophrenia (Mirmics et al., Trends Neurosci., 2001, 24, 479-486)

STOP deficient or control wild type (WT) female mice, nulliparous, 8 weeks old, were treated either with a placebo (carrier alone), or with epothilone D. Epothilone D was injected intra-peritoneally, in two injections a week, at a total dose of 4 mg/kg/week, for 8 weeks. The drug was diluted at a final concentration of 0.2mg/ml in water, from a 50mg/ml stock solution in DMSO.

For test of the effect of epothilone, we examined mouse spontaneous activity, and mouse ability to build a nest and to retrieve pups. Whereas behavioral defects in STOP-/- mice are complex, these defects ultimately result in conspicuous alterations of spontaneous behaviour, with a fragmented activity characterized by frequent shifts between activities, and in severe deficits affecting tasks related to nurturing, such as nest building and pup retrieving.

Spontaneous activity was recorded and quantified during a three hours period of time, as in Andrieux et al. Five activities were considered: feeding, remaining still without sleeping, walking, grooming and sleeping. For each activity, the total time spent doing the activity and the number of distinct sequences of activity, were determined.

For assessment of nesting capacity, the tested mouse was placed in a 240x240x120 mm cage containing litter and provided with a Kleenex tissue folded in 4 (final dimensions, 100x100 mm). After 60 hours, the mouse ability to use the paper and to build a nest was scored as follows:

Tissue use score: 0, the Kleenex tissue remained folded; 1, the tissue has been unfolded but not shredded; 2 the tissue was shredded.

Nest building score: 0, no attempt to build a nest; 1, primitive flat nest of uncontrolled shape; 2,true nest, the paper is mixed with litter to form a circular nest, less than 80mm in diameter.

For assessment of retrieving: following the nest building test, mice were trained and assessed for pup retrieving as in Andrieux et al.. Retrieving was scored as the number of pups retrieved (0 to 3).

Finally a global score for nesting and retrieving capacity (TNR) was determined for each mouse, by adding the tissue use, nest building, and pup retrieving scores.

Among 19 STOP -/- mice, 9 were treated with placebo injections, 10 with epothilone D. Among 20 WT mice, 10 were treated with placebo injections and 10 with epothilone D.

In STOP deficient mice, epothilone treatment caused a remarkable decrease in the total number of shifts between activities (see figure 1). This decrease in number concerned the number of walking, and grooming sequences, whereas the number of sleeping and feeding sequences remained unaffected. The total time spent grooming was also highly significantly diminished by epothilone treatment. Finally, epothilone treatment tended to diminish the time spent remaining still without sleeping, and to increase the time spent sleeping. These results indicate a conspicuous alleviation of the activity fragmentation that is characteristic of untreated STOP deficient mice (Andrieux et al.), with a trend to diminish abnormal activities such as remaining still without sleeping, or activities that can be stereotypic such as grooming, and to increase sleeping which is deficient in untreated STOP-/- mice (Andrieux et al.).

In the same STOP deficient mice, epothilone treatment strongly improved the paper use score, the nest building score, and tended to improve the pup retrieving score. There was a highly significant increase in the TNR score, which rose from 1.16 to 4.4, upon epothilone treatment, in STOP-/- mice (Figure 2). The treatment thus had a remarkable beneficial effect on nurturing-related tasks that are strongly deficient in untreated STOP-/- mice.

In contrast, in WT mice, epothilone treatment had no significant effect on the recorded activities or on nurturing-related behaviours, with the exception of a significant increase in the number of sequences of stillness (Figure 1). However, the observation of a single significant difference among multiple comparisons is compatible with random fluctuations. Altogether, results indicate that epothilones have little or no psychotropic effects in WT mice.

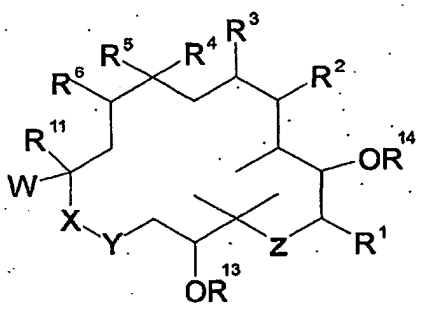
Taken together, these results show that epothilone treatment can alleviate behavioural disorders in an animal model of psychiatric disease involving connectivity disorders.

CLAIMS

1. Use of at least one epothilone or derivative thereof as an active ingredient for manufacturing a medicament for use in the treatment of disease(s) involving a neuronal connectivity defect.

2. Use of at least one epothilone or derivative thereof as an active ingredient for manufacturing a medicament for use in the treatment of schizophrenia or autism.

3. Use according to claim 1 or 2, wherein the epothilone is a compound of formula (I):



(I)

wherein:

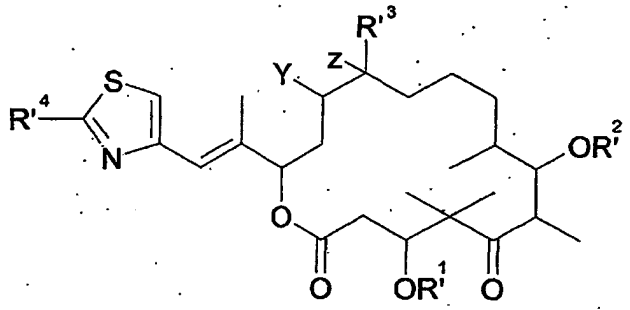
- R^1 represents H, alkyl, alkenyl or alkynyl in C_1-C_6, aryl in C_6-C_{10}, aralkyl in C_7-C_{15},
- R^2, R^3 represents each H or form together C=C double bond,
- R^4 represents C_1-C_6-alkyl in particular CH_3, fluoro substituted C_1-C_6 alkyl in particular CF_3 or CFH_2,
- R^5 and R^6 form a C=C double bond or a three membered ring including O, S, NR^7, CR^8R^9 with R^7 being $C(O)R^{10}$, SO_2R^{10} and R^8, R^9, R^{10} being independently H, halogen, C_1-C_6 alkyl, C_6-C_{10} aryl, C_7-C_{15} alkaryl,
- R^{11} being H, C_1-C_6 alkyl, C_6-C_{10} aryl, C_7-C_{15} alkaryl, and in particular H,
- W represents $C(R^{12})$=CH, $C(R^{12})$=CF or a bicyclic aromatic/heteroaromatic radical preferably a 2-methylbenzothiazol-5-yl radical, or a 2-methylbenzoxazol-5-yl radical or a quinolin-7-yl radical, with R^{12} representing a heteroaromatic radical, preferably a 2-pyridinyl, a 2-substituted thiazol-4-yl or a 2-substituted oxazol-4-yl radical with substitution in 2-position by C_1-C_6 alkyl,

pseudohalogen like CN or N_3, S-C_1-C_4-alkyl, O-C_1-C_6-alkyl, or C_1-C_6-alkyl substituted by OH, amino, halogen, pseudohalogen such as –NCO, -NCS, $–N_3$, O-(C_1-C_6)-acyl, O-(C_1-C_6)-alkyl or O-benzoyl,

- X-Y represents O-C(=O), O-CH_2 , CH_2-O, CH_2-C(=O),
- Z represents C=O, S, S=O, SO_2,
- R^{13} and R^{14} represents independently from each other H, C_1-C_6-alkyl, (CO)R^{15} or C_{1-4}-trialkylsilyl, with R^{15} being H, C_1-C_6-alkyl, fluoro substituted C_1-C_6-alkyl,

and pharmaceutically acceptable salts thereof.

4. Use according to any one of claims 1 to 3, wherein the epothilone is a derivative of following formula (II):



(II)

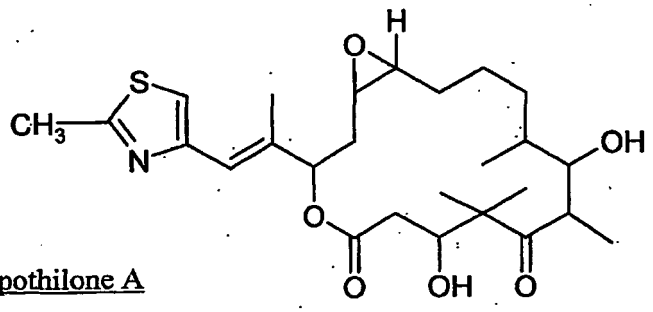
wherein:

- R'^4 represents an C_1-C_6 alkyl or substituted C_1-C_6 alkyl with substituents as F, Cl, Br or I, pseudohalogen, such as –NCO, -NCS , $–N_3$, NH_2, OH, O-(C_1-C_6)-acyl, O-(C_1-C_6)-alkyl or O-benzoyl,
- R'^1 and R'^2 are independently from each other H, C_1-C_6-alkyl, (CO)R'^5 with R'^5 being H, C_1-C_6-alkyl, C_1-C_6-fluoroalkyl or C_{1-4}-trialkylsilyl,
- R'^3 represents H, C_1-C_6-alkyl, halogen substituted C_1-C_6-alkyl, and
- Y and Z form either a C=C double bond or are the O atom of an epoxide

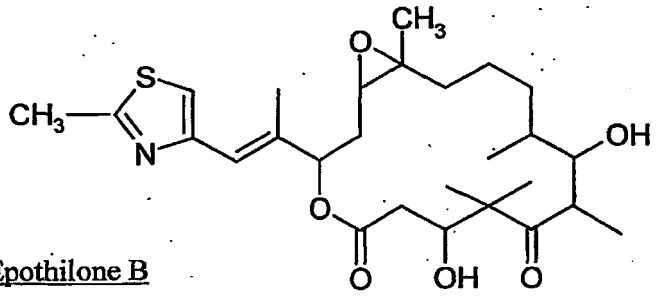
and pharmaceutically acceptable salts thereof.

5. Use according to claim 4, wherein the epothilone is at least a derivative of formula (II) wherein R'^1, R'^2, R'^3 represents independently from each other, H, C_1-C_6-alkyl in particular CH_3, C_1-C_6 fluoroalkyl in particular CF_3 and Y and Z form either a C=C double bond or are together the O atom of an epoxide.

6. Use according to any one of claims 1 to 5, wherein epothilone includes at least the natural epothilone A or B of following formula:



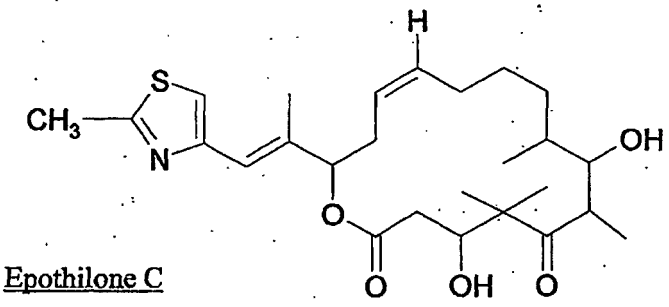
Epothilone A



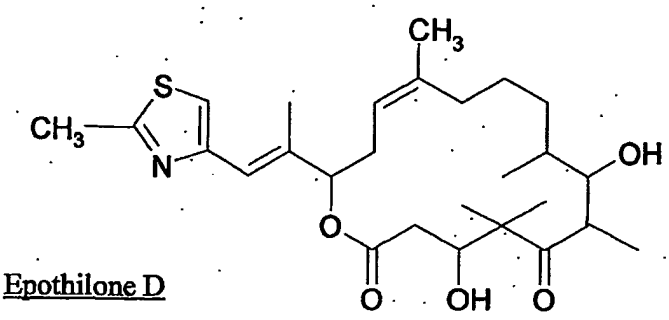
Epothilone B

or a pharmaceutically acceptable salt thereof.

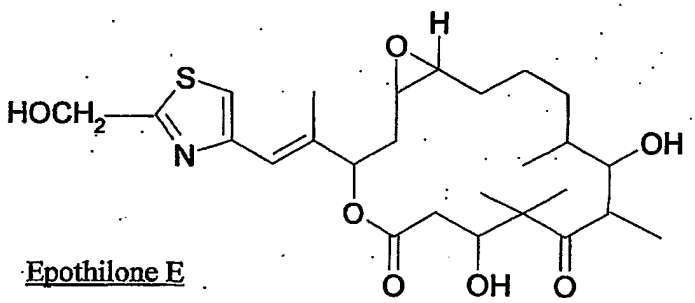
7. Use according to any one of claims 1 to 6, wherein epothilone includes at least one synthetic epothilone C, D, E or F of following formula:



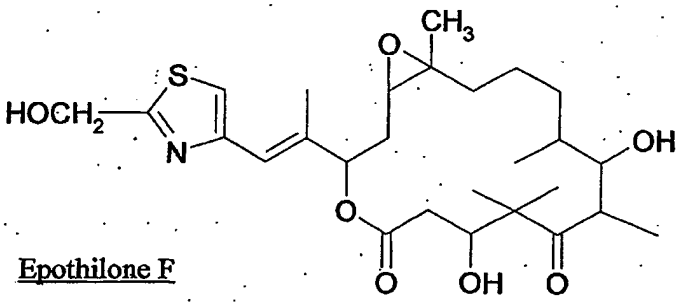
Epothilone C



Epothilone D



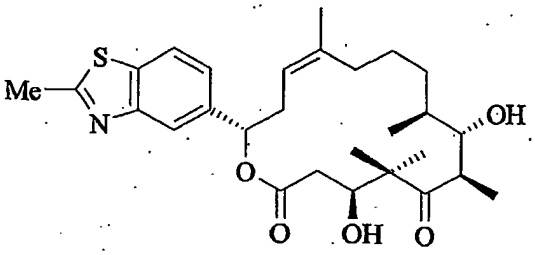
Epothilone E

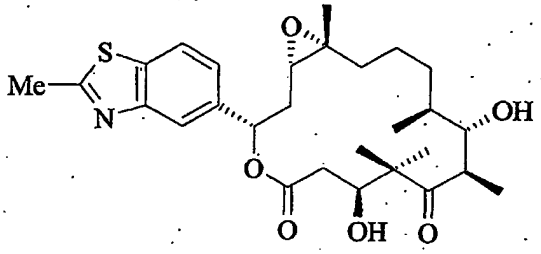


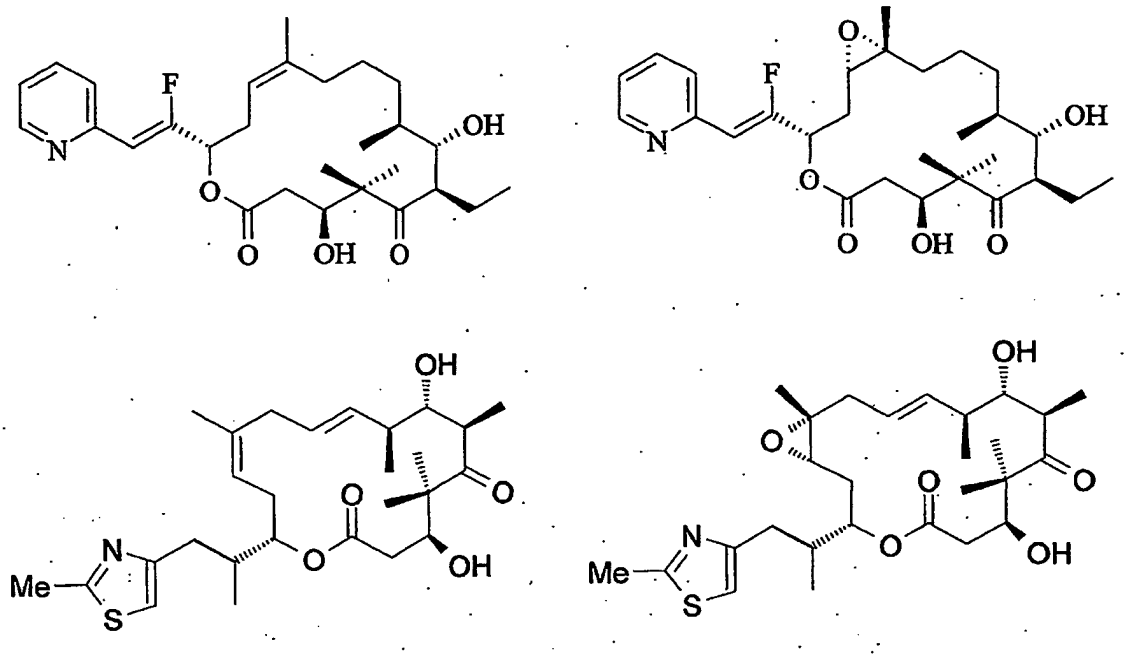
Epothilone F

in particular epothilone D and pharmaceutically acceptable salts thereof.

8. Use according to any one of claims 1 to 7, wherein epothilone includes at least one synthetic epothilone of following formula:







9. Use according to any one of claims 1 to 8, wherein the epothilone(s) is used at a therapeutically effective amount from about 0.01/Kg/dose to about 100 mg/Kg/dose.

10. Method of treatment of a disease involving a neuronal connectivity defect comprising administering to an individual in need thereof a therapeutic effective amount of one epothilone or derivative thereof.

11. Method of treatment of a disease involving a neuronal connectivity defect comprising administering to an individual a therapeutically effective amount of at least one epothilone or derivative thereof in a pharmaceutical composition comprising at least a pharmaceutically acceptable carrier.

12. Method according to claim 10 or 11, wherein the disease includes a psychotic or psychiatric disorder.

13. Method according to any one of claims from 10 to 12, wherein the epothilone or pharmaceutical compositions thereof is administered in combination with one or more agents useful in preventing or treating psychotic or psychiatric disorders.

14. Method according to any one of claims from 10 to 13, wherein the epothilone is as defined in claims 3 to 9.

ABSTRACT

INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE

« Use of epothilones in the treatment of psychotic disorders with neuronal connectivity defects »

The present invention is about the use of at least one epothilone or derivative thereof as an active ingredient for manufacturing a medicament for use in the treatment of disease(s) involving a neuronal connectivity defect.

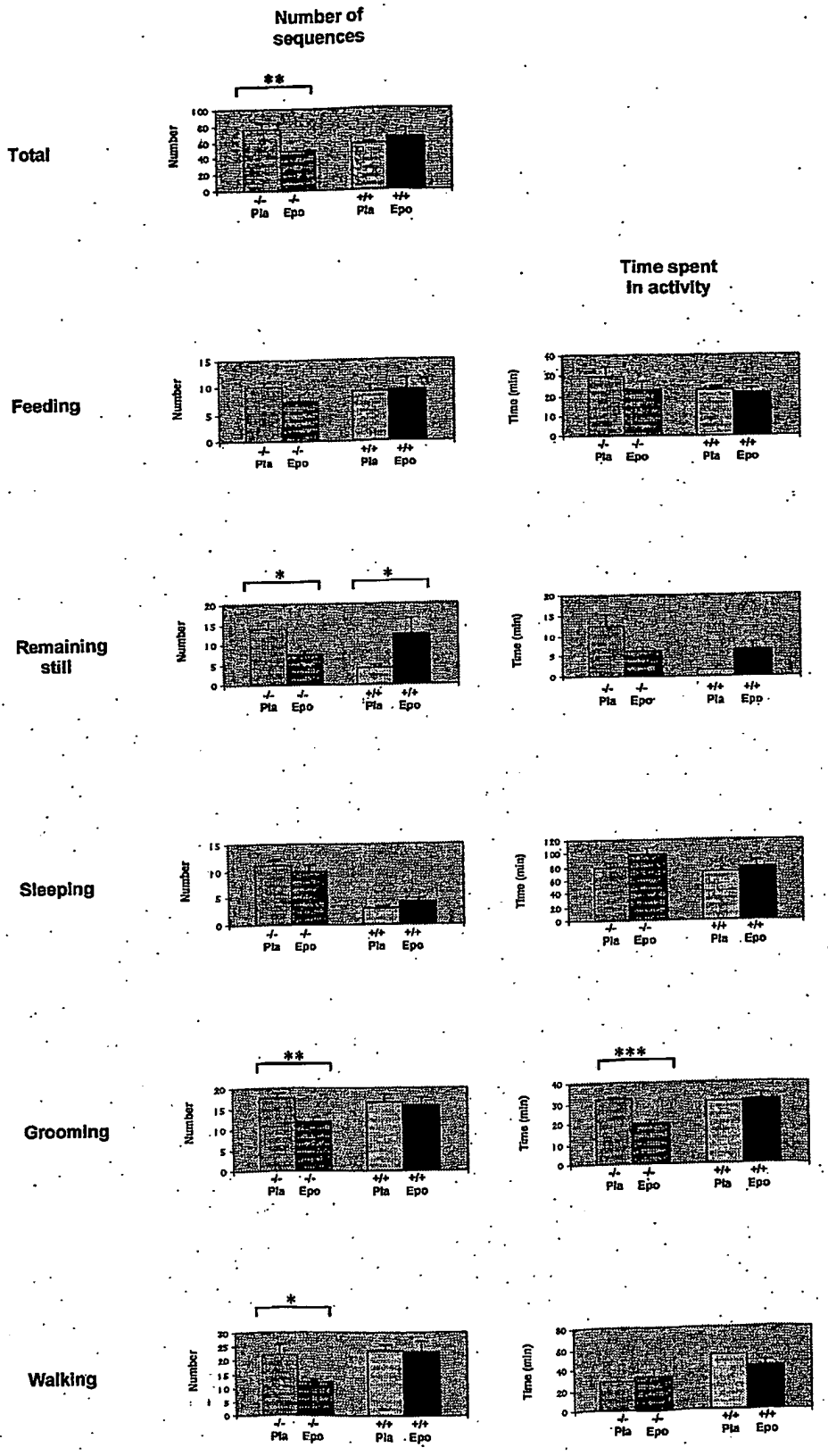

Number of sequences
Time spent in activity
Total
Feeding
Remaining still
Sleeping
Grooming
Walking
Number
Time (min)
-/- Pla
-/- Epo
+/+ Pla
+/+ Epo
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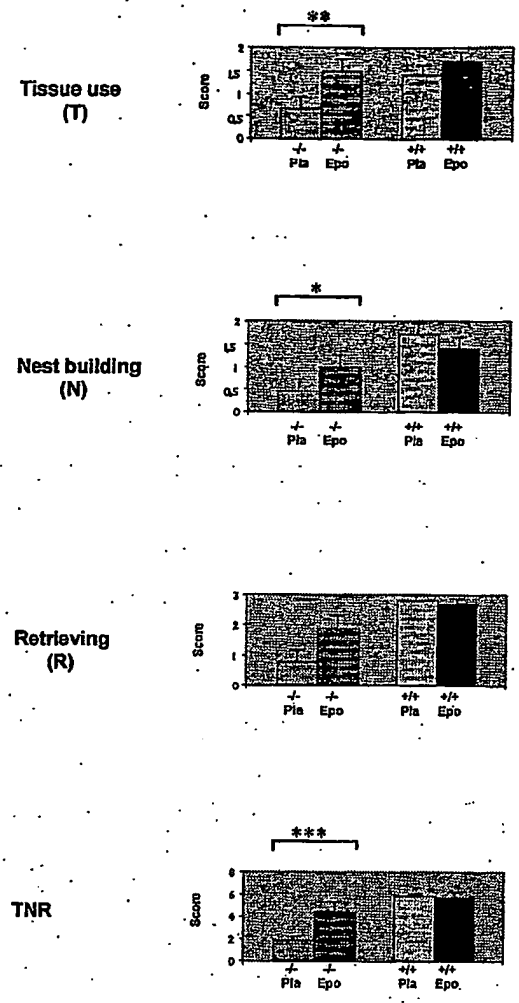
FIGURE 1


Tissue use
(T)
**
Score
-/- Pla
-/- Epo
+/+ Pla
+/+ Epo
Nest building
(N)
*
Score
-/- Pla
-/- Epo
+/+ Pla
+/+ Epo
Retrieving
(R)
Score
-/- Pla
-/- Epo
+/+ Pla
+/+ Epo
TNR
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Score
-/- Pla
-/- Epo
+/+ Pla
+/+ Epo

FIGURE 2